Design and Synthesis of Planar Telomestatin Analogs

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Martin S. Seyfried

von Zürich und Meilen

Promotionskomitee

Prof. Dr. Nathan W. Luedtke (Vorsitz)

Prof. Dr. Stefan Bienz

Prof. Dr. John A. Robinson

Zürich 2011

.

Design and Synthesis of Planar Telomestatin Analogs

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Martin S. Seyfried

von Zürich und Meilen

Promotionskomitee

Prof. Dr. Nathan W. Luedtke (Vorsitz)

Prof. Dr. Stefan Bienz

Prof. Dr. John A. Robinson

Zürich 2011

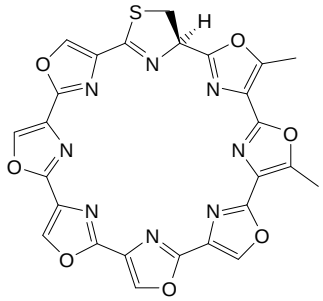
To my family

For their constant encouragement, support and guidance
throughout my entire education

Table of contents

1. Zusammenfassung

Telomestatin (*Abb. 1.1*) ist ein von *Streptomyces anulatus* 3533-SV4 isolierter Naturstoff. Es ist einer der stärksten Inhibitoren für das Enzym Telomerase und weist auch sonst ein breites Spektrum biologischer Wirkungen auf.

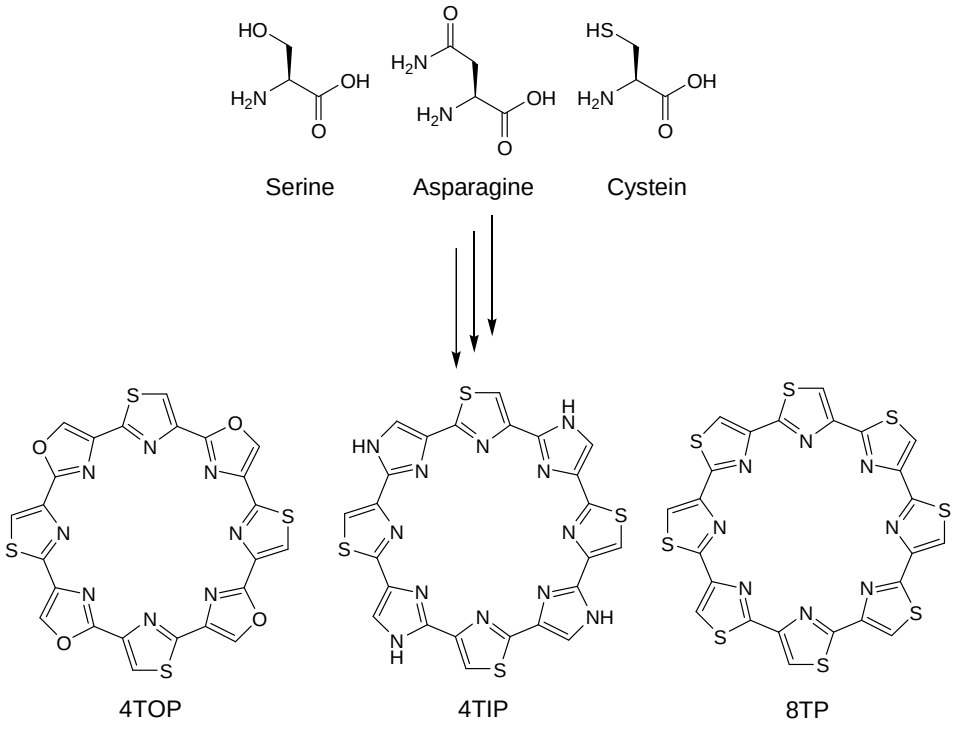


Telomestatin

Abb. 1.1: *Der Naturstoff Telomestatin*

Man vermutet, dass Telomestatin über eine selektive Bindung an die G-Quadruplex Struktur wirk. Diese ist u.a. im 3'-Überhang der Telomerenenden menschlicher Chromosomen zu finden. Die hohe Affinität und Spezifität für diese G-Quadruplex DNS erklärt man sich mit der zum G-Quartet der G-Quadruplex DNS gut passenden Grösse und Form des Moleküls. Die Form von Telomestatin ist jedoch auf Grund der einen Thiazolin-Einheit nicht ideal, da diese die Planarität von Telomestatin aufhebt. Trotz aller Versuche ist es bisher niemandem gelungen, einen Makrozyklus herzustellen, der acht Azol-Einheiten enthält und vollständig planar ist. Der Grund dafür liegt vermutlich an einer erhöhten makrozyklischen Ringspannung. Diese sollte durch Substitution von vier Oxazol- durch vier Thiazol-Einheiten im makrozyklischen System reduziert werden können. Daher arbeiteten wir an der Synthese von drei neuen, vollständig planaren Telomestatin Analoga, die jeweils vier Thiazole und entweder vier Oxazole, Imidazole oder Thiazole enthalten (*Schema 1.1*). Die folgende Arbeit berichtet von der Konzipierung und der synthetischen Arbeit hin zu diesen Molekülen, wobei die drei Aminosäuren Serin, Asparagin und Cystein als Startmaterialien dienten (*Schema 1.1*).

Schema 1.1. *Synthese neuer Telomestatin Analoga*

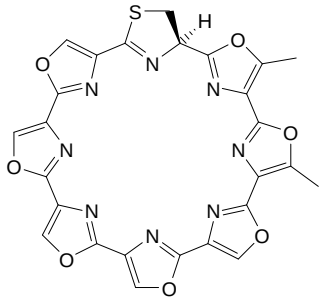


Im Prinzip wurden zwei Aminosäuren miteinander gekoppelt, worauf der Sauerstoff der neu gebildeten Amidbindung und die Seitenkette der einen Aminosäure unter Wasserabspaltung zum Azol zyklisiert wurden. Die synthetisierte, Azol enthaltende neue Aminosäure wurde tetramerisiert und makrolaktamisiert, wobei Standard Peptidchemie angewendet werden konnte. Die dabei übrig bleibenden Aminosäuren Seitenketten wurden anschliessend wiederum unter Dehydratatisierung für Ringschlüsse verwendet. So konnte in 18 Synthesestufen das C_4-symmetrische 4TOP hergestellt werden. Dieses ist die erste, acht Azole enthaltende, makrozyklische Verbindung, die je hergestellt worden ist.

Auf dem Weg zu diesem anspruchsvollen Ziel, konnten neue synthetische Methoden entwickelt werden. Es ist dies zum einen die Verwendung von Silyl-geschützten Verbindungen für Zyklodehydratatisierungen, sowie eine sehr effiziente und milde Methode, geschützte Aminosäuren mit ^{18}O zu markieren.

2. Summary

Telomestatin (*Figure 2.1*) is a natural product isolated from *Streptomyces anulatus* 3533-SV4. It is one of the most potent telomerase inhibitors reported to date, and it exhibits a broad range of biological activities.

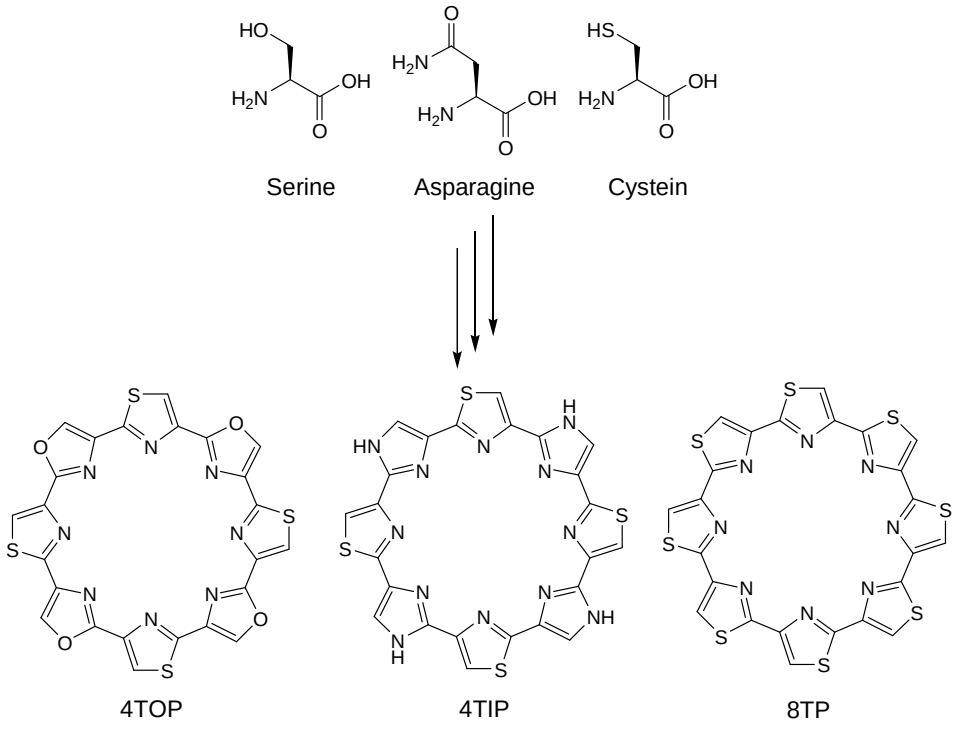


telomestatin

Figure 2.1: *Natural product telomestatin*

The proposed mode of action of telomestatin involves selective binding of G-quadruplex structures present in the 3' telomeric overhang of human chromosomes. The high affinity and specificity of telomestatin for G-quadruplex DNA is rationalized by its size and shape complementarity with the G-tetrads of G-qadruplex DNA. In reality, the shape of telomestatin is not perfect for G-tetrad stacking due to the presence of a single thiazoline unit. This makes telomestatin a non-planar molecule. Due to high macrocyclic ring-strain, all attempts to synthesise a macrocycle containing eight azole-units have thus far proven fruitless. Ring-stain is reduced by replacing four oxazole units with thiazole in the macrocyclic system. We therefore pursued the synthesis of three new, fully planar telomestatin analogs, each containing four thiazoles and the remaining units comprising oxazoles, imidazoles or thiazoles (*Scheme 2.1*). The following work presents the design and the synthetic efforts towards these target molecules starting with three different amino acids: serine, asparagine and cysteine (*Scheme 2.1*).

Scheme 2.1. *Synthesis of new telomestatin analogs*



Our synthetic strategy involves the coupling of amino acids followed by cyclodehydration reactions. The resulting azole-containing amino acids are tetramerized and macrolactamized using standard peptide chemistry. The remaining amino acid side chains are then used for four cyclodehydration reactions in a single step. In 18 synthetic steps total, the C_4-symmetric 4TOP could be synthesised. This is the first macrocyclic compound containing eight azole units ever reported.

Along the way towards this challenging goal, new synthetic methodologies were developed including the use of silyl-protected alcohols for cyclodehydration reactions, as well as a highly efficient and mild method for ^{18}O labelling of protected amino acids.

3. Introduction

3.1 G-Quadruplex DNA

3.1.1 Structure

In 1910, Bang and coworkers observed that guanosine monophosphate (GMP) behaves differently than other known nucleotides, due to self-assembly of GMP that results in a gel[1]. More than 50 years later, Gellert, Lipsett and Davis[2] proposed an explanation for this phenomenon, where four guanines arrange themselves in a square planar array with two hydrogen bonds on the face of each guanine residue. This type of "Hoogsteen" basepairing was predicted by Karst Hoogsteen in 1963. Such G-tetrads are further stabilized by monovalent cations such as K^+ between two stacks of G-tetrads (*Figure 3.1*). Multiple stacks G-tetrads are contained within four-stranded G-quadruplex structures. The thermodynamic stability of G-quadruplexes is comparable to duplex DNA[3] and include similar stabilizing contributions like π-stacking, hydrophobicity and dispersion effects[4]. However, the unfolding kinetics of G-quadruplexes are very slow compared to DNA or RNA hairpins[3].

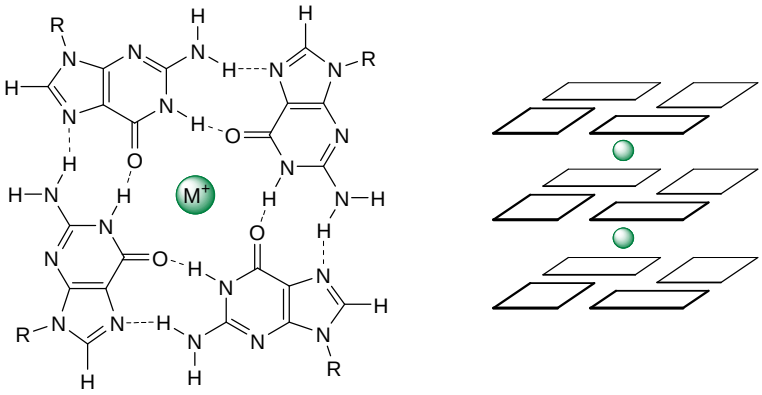


Figure 3.1: *Four guanines can form a G-tetrad and multiple stacks of G-tetrads form a G-quadruplex*

Described around the same time as Watson and Crick proposed their famous DNA model for the B-form duplex, G-tetrads remained a laboratory curiosity for many decades. It changed with the finding of genomic sequences with the ability to form G-quadruplexes under physiological conditions[5, 6]. This has motivated intensive investigations of G-rich sequences and their associated secondary structures, rapidly becoming a "hot topic" in current DNA research.

G-quadruplexes can be formed from one, two or four different DNA- or RNA-strands, and display a wide variety of topologies with variable orientations and differing connectivity of the strands. Variable loop arrangements also contribute to the characteristic 3-dimensional structures.

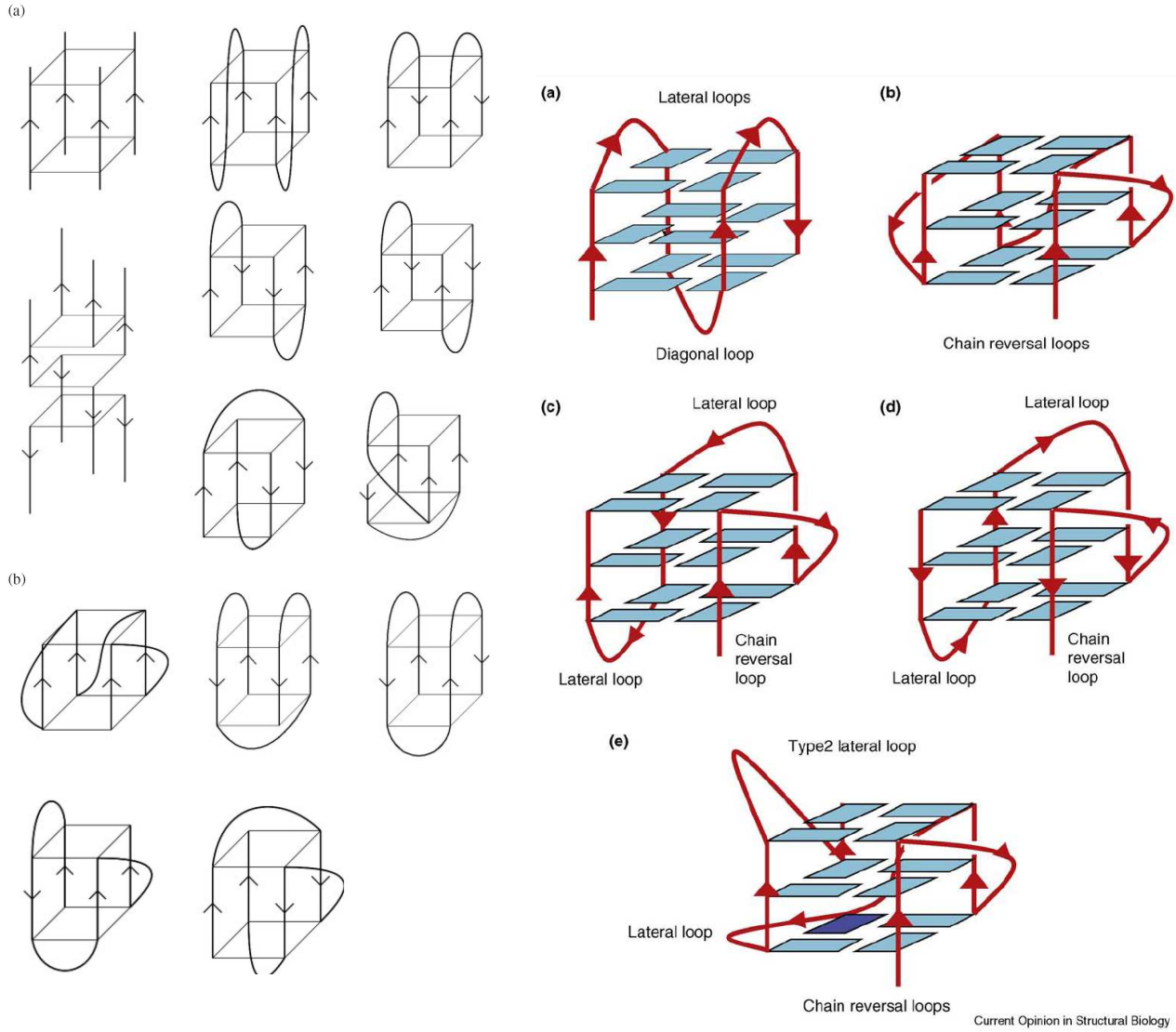


Figure 3.2: *left side: some different topologies of G-quadruplexes resulting from different numbers and orientations of DNA-strands[7], right side: different common names for loop topologies including lateral, diagonal or chain reversal loops[8].*

There are three main categories of G-quadruplexes including "intramolecular" structures that have a putative quadruplex sequence of $G_mX_nG_mX_oG_mX_pG_m$ with m as the number of G-residues directly involved in G-tetrad interactions. X_n, X_o and X_p can be combinations of any residues of unequal length forming the loops between the G-tracts. The second and third categories are the bimolecular (or dimeric) and the tetramolecular (tetrameric) quadruplexes, where the G-tracts are divided into two or four separate oligonucleotide chains. Quadruplexes are also classified according to

their strand polarties when possible. For example, adjacent parallel strands can be termed a "parallel" G-quadruplex. For intramolecular structures, these require connecting loops that link the G-tetrads with chain reversal loops, also called "propeller" type loops. In cases where one of the G-tracts is oriented in an anti-parallel fashion the whole structure can be designed as an "anti-parallel" G-quadruplex. This type of topology is commonly found in bimolecular quadruplexes and in many of the unimolecular structures determined to date[7, 9]. These topologies require one or more lateral loops, also termed as "edge-wise" loops. Another possible loop is the "diagonal loop", capping the G-quadruplex and connecting two opposite tracts in the tetrad.

In parallel G-quadruplexes, guanosine glycosidic angles are normally all in an *anti* conformation. Anti-parallel quadruplexes typically have a mixture of both, *syn* and *anti* guanosines, arranged in a characteristic pattern for each structure[7].

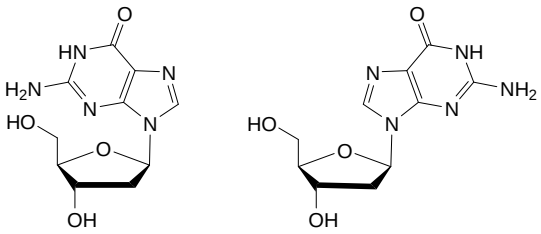


Figure 3.3: syn- *and* anti-*guanosine*

All G-quadruplexes have four grooves that are defined by the phosphodiester backbones. Groove dimensions are highly variable and depend on the overall topology of the quadruplex, strand orientation and nature of the loops.

The stability of G-quadruplexes in H_2O is highly dependent on the presence, concentration and nature of the cations incorporated between layers of G-tetrads. This is a result of the strong negative electrostatic potential from the guanine O^6 oxygen atoms that all point towards the centre of the G-tetrad, as well as the global electrostatic field of the anionic phosphates concentrated in the geometric centre of the structure. Central positioning of cations minimizes electrostatic repulsion. In the case of Na^+, the position of the ion can differ from structure-to-structure, with sodium ions lying either in the plane of one G-tetrad, or between two successive G-tetrads. K^+ ions, in contrast, are always positioned in between two planes defined by two G-tetrads. G-quadruplexes are better stabilized by K^+ as compared to Na^+ ions due to their lower desolvation energies[10].

3.1.2 Potential biological relevance

Sequences with the potential to form G-quadruplex structures can be found throughout the genome[11, 12]. Huppert and Balasubramanian estimated that 376'000 putative G-quadruplex sequences $G_mX_nG_mX_oG_mX_pG_m$ are present in the human genome, somewhat less than expected by random chance[11, 13]. These sequences are mainly found in telomeric regions and in transcriptional regulatory sites. While evidence for the existence of G-quadruplex structures in multicellular organisms is still sparse, a growing number of studies provide indirect evidence for potential biological relevance. G-quadruplexes are possibly involved in the regulation of telomeric length[14-16] and may play a role in the regulation of gene transcription[17, 18], and may serve as a trigger for somatic recombination[19-21]. Binding interactions between G-quadruplex-forming sequences and nucleolin possibly influence the transcription of rDNA and the processing of rRNA[22].

3.1.2.1 Telomeres

The main function of telomeres includes the protection of chromosomal ends from recombination, degradation and end-to-end fusions. This is achieved by a protein-DNA complex termed "shelterin"[23, 24]. Telomere stability and regulation are also related to the end replication problem. Due to RNA-primed DNA synthesis, telomeres get shorter with each round of cell division, until they become too short and loose their capping function. This can result in chromosome fusion, cell senescence and/or apoptosis. The end replication problem can therefore limit the number of cell divisions a somatic cell can undergo. Stem cells and other immortalized tissues require a mechanism to correct this loss. Most immortal tissues, including ~80% of cancers overexpress a ribonucleoprotein complex called telomerase that is able to elongate the telomeres using a RNA template and reverse transcriptase activity. Molecules known to bind to and to stabilize G-quadruplexes *in vitro* can reduce the activity of telomerase in cells[23, 25].

The telomeres of vertebrates contain repetitive units of $d(GGGTTA)_n$-3'. A human telomere has approximately 1000 copies of this unit[26, 27] and ends with a single stranded 3'-overhang of 100-200 nucleotides[24, 28]. This sequence is capable of forming stable G-quadruplexes *in vitro*[29, 30]. The potential abilities of telomeric structures to prevent degradation, fusion or other damage remains largely speculative[24]. One potential mechanism is the formation of so called "t-loop" or "D-

loop" type structures where the single stranded 3’ end is integrated into the terminal duplex region of the double stranded telomeric DNA. This might result in a triple stranded region containing a G-quadruplex formed by the displaced strand. Many proteins are associated with the telomeric "shelterin" complex. These include the POT1 protein that can bind to the single stranded 3’ overhang, while TRF1 and TRF2 proteins bind to the double stranded telomeric DNA[31]. Studies on POT1 showed that this protein disrupts the formation of a G-quadruplex structure by binding the single-stranded 3’ overhanging telomeric sequence[32]. Interestingly, the treatment of human EcR293 cells by a selective G-quadruplex binding molecule "telomestatin" induces a dramatic and rapid delocalization of POT1 from its normal telomere sites, but telomestatin does not affect the telomere localization of TRF2. This suggests that the overhang can exist in either POT1-bound or G-quadruplex forms[33]. The maintenance of proper telomere stability likely requires a dynamic cap that allows access to telomeric DNA during synthesis and telomeric extension.

3.1.2.2 Promoters

G-quadruplex forming motifs have been found in the promoter regions of a large number of human oncogenes like c-MYC, VEGF, HIF-1a, Ret, KRAS, Bcl, c-Kit, PDGF-A and c-Myb. Genomics analyses have revealed that 43% of the human gene promoters contain a putative G-quadruplex forming sequence $G_mX_nG_mX_oG_mX_pG_m$[13]. This is 6.1 times more frequently than it would be expected for genomic DNA[34]. The enrichment of G-quadruplex motifs is highest close to the transcription start site (TSS), while relatively few of these motifs are found in exons. Genes involved in cancer evolution and growth are even more likely to have promoter G-quadruplex motifs with approximately 67% of these promoters containing a G-quadruplex motif according to Huppert and Balasubramanian.

In 2007 Johanna Eddy and Nancy Maizels published their research on potential G-quadruplex forming sequences in the -1000 to +1000 bp surrounding the TSS and addressed the possible contributions of the template and nontemplate strand[35]. They suggested, that G-richness upstream from TSS can be accounted for by well-defined functional motifs in duplex DNA including "CpG-islands" for methylation[36, 37] as well as G-rich motifs recognized by well-characterized transcription factors that are specific for duplex DNA, including SP1, KLF, EKLF, EGR-1 and AP-2[38-40]. Downstream of the TSS they found many other human genes containing G-rich

putative G-quadruplex-like motifs, located on the nontemplate strand on the 5'-end of the first intron (referred to as 'GrIn1'). These sequences could not be explained as sequences recognized by known transcription factors such as those mentioned above. An estimated 8% of all human genes contain a GrIn1-type sequence. The authors speculated that these sequences, being located on the nontemplate strand, potentially can play a role in the regulation of transcription when forming G-quadruplex structures following RNA polymerase denaturation of duplex DNA or subsequently during splicing of the pre-m-RNA.

One of the most investigated systems is c-MYC, an important oncoprotein and transcriptional factor that plays a central role in cell proliferation and induction of apoptosis. Overexpression of c-MYC is associated with a significant number of human malignancies like breast, colon, cervix and small-cell lung cancers. The control of c-MYC expression is mediated by many different proteins and DNA sequences, including the nuclease hypersensitivity element III_1 (NHE III_1) that is located between -142 and -115 base pairs upstream of the c-MYC transcription start site. It has been claimed that NHE III_1 controls up to 80-90% of the transcriptional activity of the c-MYC gene[41-45]. Studies by Simonsson and his group showed that this G-rich strand can readily form a G-quadruplex structure *in vitro* under physiological conditions and that the formation of this structure is K^+ dependent[46]. Experiments by Hurley and co-workers suggest that this G-quadruplex can have a regulatory effect on transcriptional initiation. In these experiments, gene expression profiles in HeLa cells were evaluated after treatment with TMPyP4, a molecule known to stabilize the G-quadruplex, and revealed downregulation of transcription. Site directed mutagenesis experiments had similar effect, where mutations that destabilized G-quadruplex formation increased gene expression. Control experiments using TMPyP2[47], a constitutional isomer of TMPyP4 showed no impact on gene expression, presumably due to the lower G-quadruplex affinities of these compounds[48]. Together these results suggest that c-MYC expression may be mediated by a G-quadruplex structure formed in the c-MYC promoter. Hurley and Qin proposed that TMPyP4 has an impact on the dynamic equilibrium between duplex, unwound single-stranded and G-quadruplex and i-motif structures[49] (*Figure 3-4*).

a) **5' – TGGGGAGGGTGGGGAGGGTGGGGAAGG – 3'**

b)

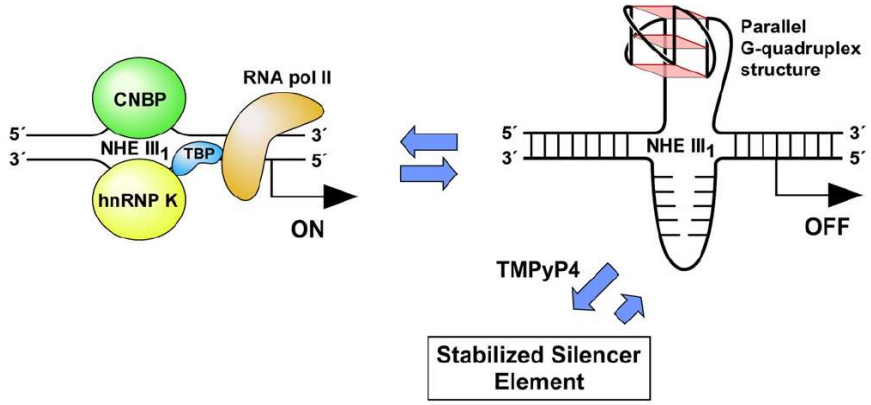


Figure 3.4: *a) sequence of the c-MYC NHE III_1 and b) proposed c-MYC promoter dynamics showing duplex DNA undergoing transcription versus a transcriptionally silent G-quadruplex in the NHE III_1 sequence*[49]

3.1.2.3 Recombination

Homologous recombination requires co-localization of homologous DNA segments. The DNA conformations associated with pre-recombination complexes remain poorly understood. Triplex-induced recombination has been studied in some detail[50], but involvement of G-quadruplex structures has only been recently proposed. In 2006 Shukla and Roy investigated the influence of a potential G-quadruplex on recombinatorial events in plasmids. They report an increasing recombination rate upon insertion of a G-quadruplex forming sequence between two direct repeats, which suggests that the formation of a G-quadruplex is able to bring the two repeats together and therefore might facilitate recombination.

In 2009 Chowdhury *et al.* published a genome-wide analysis of recombination prone regions to predict the role of DNA structural motifs in recombination[21]. They found that potential G-quadruplex DNA forming sequences were significantly enriched near hotspots of recombination as compared to AT-rich DNA regions. They found that within a 50 base pairs of putative G-quadruplex DNA sequences, there is an enrichment of previously reported short sequence motifs known to be recombination hotspots (like for example, the long terminal repeat of two retrovirus-like retrotransposons, THE1A and THE1B, or CT- and GA-rich repeats[51]). There was also a significant co-localization of quadruplex motifs with target sites for three known transcription factors: c-Rel, NF-kappa B (p50&p65) and Evi-1[21]. Based on these findings, Chowdhury *et al.* proposed that G-quadruplex formation could be one

of the key determinants of recombination where the single stranded "fold back" structure could assist in strand separation and homologous pairing.

A seminal paper proving a relationship between putative quadruplex sequences and recombination was recently published by Cahoon an Seifert in *Science*[52]. Their model system was the pilin antigenic variation (AV) in *Neisseria gonorrhoeae*. Hyper-variation of surface proteins is a mechanism by which the bacterium can protect itself from the host's immune defenses. There are 46 predicted G-quadruplex forming sequences in the genome of this gram-negative bacterium and one of them seems to be closely associated with antigenic variation. One such G/C-rich sequence (3'-TTTTA AG**GGG T GGG TT GGG T GGG** A TAAGA-5') is located upstream of *pilE*, the gene encoding for the variable pilin proteins. This sequence was shown *in vitro* to form G-quadruplexes and mutations in this specific G-quadruplex sequence, disrupting the formation of the G-quadruplex structure, resulted in no more AV. Growing the bacteria at a non-toxic concentration of *N*-methyl mesoporphyrin IX (NMM), a molecule stabilizing the G-quadruplex structure, a significantly decreased "pilus phase" and AV frequency were observed. These results suggest that formation of the *pilE* G-quadruplex structure is required for wild-type pilin AV. It is likely that the structure forms only when the DNA duplex is melted, possibly during DNA replication or transcription.

3.1.2.4 Ribosomal DNA

In all eukaryotes the entire transcribed region of the rDNA is very rich in guanine residues accounting for 34% of human rDNA. The guanines are concentrated within the "spacer" regions, as well as the regions that template the mature rRNAs. These G-rich sequences are consistently located on the non-template strand, and most of them are within runs of three or more consecutive G's. rDNA is transcribed in an extraordinary high frequency. According to electron microscopic analysis[53] the polymerases follow each other with spaces of only about 100 base pairs between them. The non-template strand must therefore be constantly denatured or stabilized in a different secondary structure due to such high transcriptional activity. Hanakaha *et al.* showed, that the rDNA-associated protein nucleolin is able to bind very strongly to various G-quadruplex sequences with a dissociation constant K_d of 1 nM[22]. The authors therefore hypothesize, that the formation of G-quadruplex-nucleolin complexes stabilizes the non-transcribed strands of rDNA. Nucleolin is known to be

abundant in the dense fibrillar component of the nucleolus, where rDNA transcription occurs. It is also abundant in the peripheral granular component of the nucleolus, where rRNA is processed. Since nucleolin shows two separable domains which can bind to G-quadruplexes, the protein is also thought to have an organising ability for G-quadruplex domains. In this way it could have a scaffolding effect helping the processing of the G-rich rRNA strand.

Other observations give further support for the formation of G-quadruplex within rDNA such as highly conserved RecQ helicases that have been found to prefer G-quadruplex forming DNA sequences as substrates. As member of this family "WRN" is predominantly localized in the nucleolus of human cells[54]. WRN helicase is known to be deficient in Werner's syndrome, a disease which leads to rapid senescence. Interestingly, shortened telomeres are also reported as a consequence of WRN helicase deficiencies[55].

3.2 G-quadruplex ligands

3.2.1 Technical definitions of affinity and specificity

Before our survey of G-quadruplex ligands, the descriptive parameters for assessing binding affinity and specificity will be discussed. These measurements can be performed *in vitro* to help evaluate whether the molecule is a candidate for more extended characterization *in vivo*.

The first key-measure is the affinity which is normally described as equilibrium constant K_a (association constant) or K_d (dissociation constant).

$$\text{DNA + ligand} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{complex}$$

$$K_d = \frac{1}{K_a} = \frac{[DNA][ligand]}{[complex]} = \frac{k_{-1}}{k_1}$$

To measure equilibrium binding constants, spectroscopic methods measuring fluorescence emission[56] or UV absorption differences are widely used. Another tool is surface plasmon resonance, where the interaction of the ligand with the surface bound DNA leads to a change in the evanescent wave of the surface that can be measured using reflectance of plane polarized light[57]. ESI-MS was also recently used to determine the kinetics of quadruplex formation and ligand binding[58].

A factor related to affinity is the potential of a given molecule to thermodynamically stabilize G-quadruplex structures. This is normally reported as the increase of the G-quadruplex melting temperature (T_m) in the presence of ligand. The T_m can be measured using different spectroscopic methods: UV absorption, fluorescence, or FRET (fluorescence resonance energy transfer) where the loss of a secondary structure leads to a decrease in acceptor fluorophore emission. Other methods include circular dichroism (CD)[59], and differential scanning calorimetry (DSC). The resulting ΔT_m is related to the difference in ligand affinity between folded and unfolded states, as well as ligand concentration.

A critical aspect that is often neglected is G-quadruplex binding selectivity or in other words how much stronger the ligand binds to the G-quadruplex structure as compared to duplex DNA or single stranded DNA. This is a very important factor since duplex DNA will be much more abundant than G-quadruplex DNA in cells. One definition of selectivity is the ratio of K_d values between of a given molecule to different DNA secondary structures. This can be accomplished by measuring the K_d values[56], or alternatively, the relative specificity can be assessed by a competition dialysis assay based on the principle of equilibrium dialysis[60-62].

In addition to thermodynamic assays, there are functional assays such as telomerase inhibition. The value describing telomerase inhibition is the IC_{50} value, defined as the concentration of the compound needed to inhibit telomerase activity by 50%. Small IC_{50} values therefore represent potent inhibition properties of the compound. The IC_{50} value is usually determined by the TRAP assay (telomere repeat amplification protocol)[63]. The process requires two steps in which first an initial telomere primer is elongated by telomerase, and second, the products of the telomerase elongation are amplified by a PCR step. This assay was originally designed for molecules binding to and inhibiting the enzyme. For molecules binding to DNA there is an additional step needed to remove ligands that can interference with the PCR process. This additional step was established by Stephen Neidle and his co-workers in 2008 and allowed the realization that all previous reported IC_{50} values overestimated the potency of reported quadruplex ligand-telomerase inhibition activities[64].

A good overview of methods used to determine the G-quadruplex affinity and specificity of small molecules is presented by Lian-quan Gu *et al.*[65].

3.2.2 Structures and affinities of G-quadruplex ligands

In 1991 Zahler and his co-workers demonstrated that potassium cations have a stabilizing effect on G-quadruplex structures and that they are able to inhibit the activity of telomerase[25]. Since then, G-quadruplexes have emerged as interesting targets for small molecule inhibitors of telomerase. Many different groups and institutions have been developing G-quadruplex ligands as potential drug candidates due to their ability to inhibit telomerase or to deactivate G-quadruplex-related gene expression. Candidate molecules can be classified in several different ways; since the rational design of G-quadruplex-interacting compounds has been guided by potential π-stacking and electrostatic interactions, Teulade-Fichou and Monchaud have cataloged them according to their charge[66]. In addition to neutral and anionic compounds, there are three categories of cationic compounds resulting from (1) *in situ* protonation of a basic functional group, (2) *N*-methylation of an aza-aromatic moiety, (3) the presence of a metal ion. The more classical approach is to categorize ligands according to the core structures, as recently done by Lian-quan Gu *et al.*[65]. A review by Nielsen and Ulven[67], in contrast, only focused on the most potent G-quadruplex ligands that are flat macrocyclic structures with a size corresponding to the G-tetrad. In their review they divided these compounds into two main groups: (1) the porphyrins and related pyrrol- and isoindole based macrocycles and (2) telomestatin and amide based macrocycles.

3.2.2.1 Various compound classes

Antraquinons are among the first quadruplex-interactive ligands ever reported. Investigations started with the compound BSU-1051 having a reported telomerase inhibitory value IC_{50} of 23 μM[68].

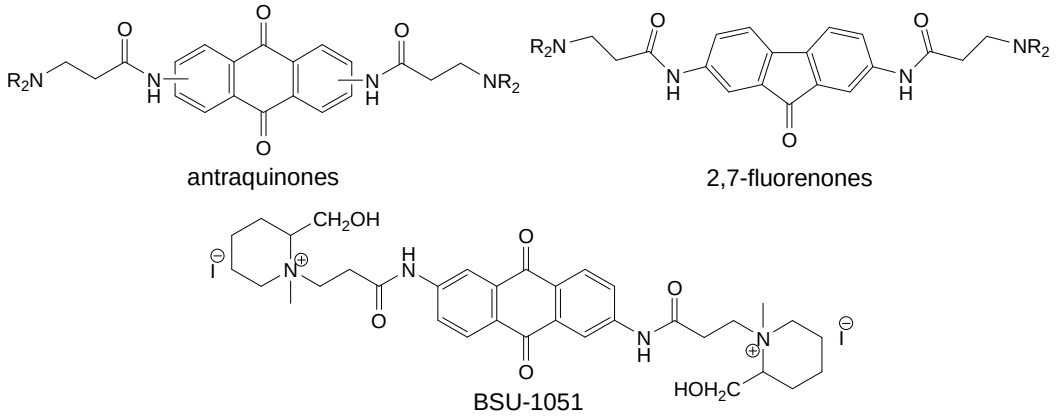


Figure 3.5: *Antraquinone G-quadruplex ligands*

A systematic structure-activity study was conducted by varying the position of the substituents and the NR_2 groups[69, 70]. The core was also exchanged with 2,7-fluorenones with the aim of reducing the cytotoxicity by lowering the potential for redox chemistry. Cytotoxicity was decreased by as much as a factor of 10 and the best IC_{50} values for telomerase inhibition were around 8-12 µM, somewhat less active than the analogous 2,7-antraquinones[71].

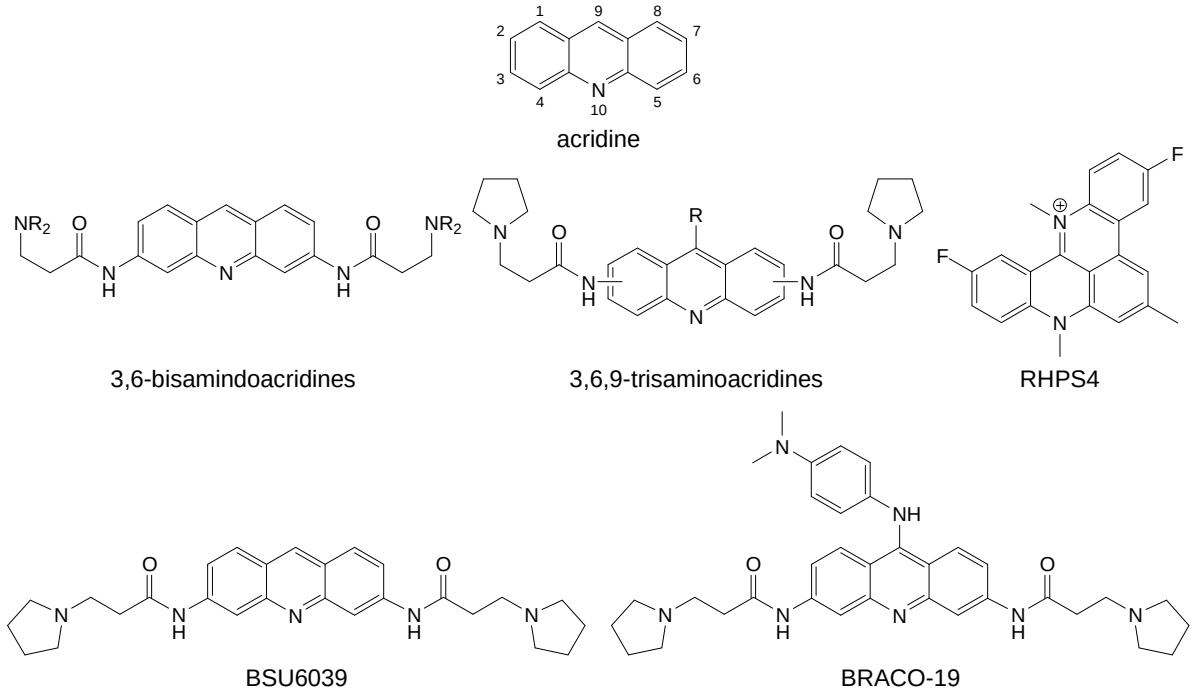


Figure 3.6: *Acridine based G-quadruplex ligands*

A related class of ligands are the **acridines**. The key difference with the antraquinons is that acridines possess a partial positive charge due to protonation of the pyridine nitrogen (pK_a of acridinium: 5.6). This charge was proposed to interact with the high electron density at the core of the G-tetrad. Various substituted

acridines were synthesised and found to have variable G-quadruplex affinity and specificity[72-74]. For example, the 3,6-disubstituted acridines show approximately the same affinity for duplex and quadruplex DNA, while the 3,6,9-trisubstituted acridines exhibit a 30-40-fold greater affinity for quadruplex DNA. One key aspect of the ligand design was a crystal structure of a G-quadruplex with BSU6039[75]. The structure showed the aromatic core stacked on the G-tetrad with the acridine side chains pointing out into the grooves. On this basis, BRACO-19 was designed, which utilizes interactions with three grooves. BRACO-19 showed good stabilization (ΔT_m = 27°C) of G-quadruplexes measured using a FRET-based assay, as well as a 31-fold preference for the G-quadruplex according to SPR experiments and a telomerase IC_{50} value of 0.15 µM. The newest ligands from this class show IC_{50} values in range of 10-20 nM[76]. An important preclinical drug candidate is the **pentacyclic acridine** 3,11-difluoro-6,8,13-trimethyl(8*H*)quino[4,3,2-*kl*]acridinium methylsulfate (RHPS4) with an IC_{50} of 0.33 µM. Interestingly, RHPS4 inhibits cell proliferation within 2-3 weeks of application at non-cytotoxic concentrations, possibly as a result of telomere destabilization[77-81]. According to NMR studies, RHPS4 binds to quadruplexes by stacking onto the external G-quartets[82, 83].

To enhance stacking interactions, the aromatic core of the acridines was extended to four fused rings. **Quindoline** and its analogs (*Figure 3-7*) bear some of the same substituents as the antraquinones and the acridines. 8-[2-(trimethylammonium) ethoxy]benzo[*b*]naphtha[2,3-*d*]furan, having a positively charged ammonium side chain, was reported to have an IC_{50} value of 7 µM[84], while the disubstituted compounds were in the range of 6 – 16 µM[85, 86].

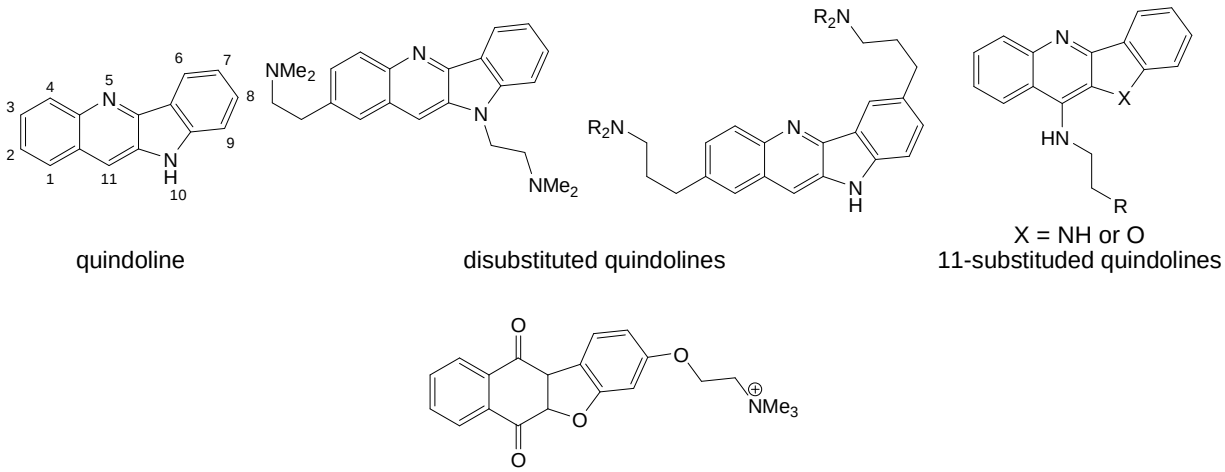


Figure 3.7: *Quindoline G-quadruplex ligands*

Quindolines having an amine at the 11-position exhibited surprisingly good telomerase inhibition activities with IC_{50} values ranging from 0.44 to 12.3 µM[87, 88]. This can be rationalized by the electron donating substituent in the 11 position which increases the basicity of the pyridine ring of the quindoline so that it is protonated under physiological conditions. These compounds were found to induce G-rich telomeric repeat sequences to fold into a quadruplex, and can also stabilize the c-MYC G-quadruplex which leads to down-regulation of c-MYC in the some cell lines[89].

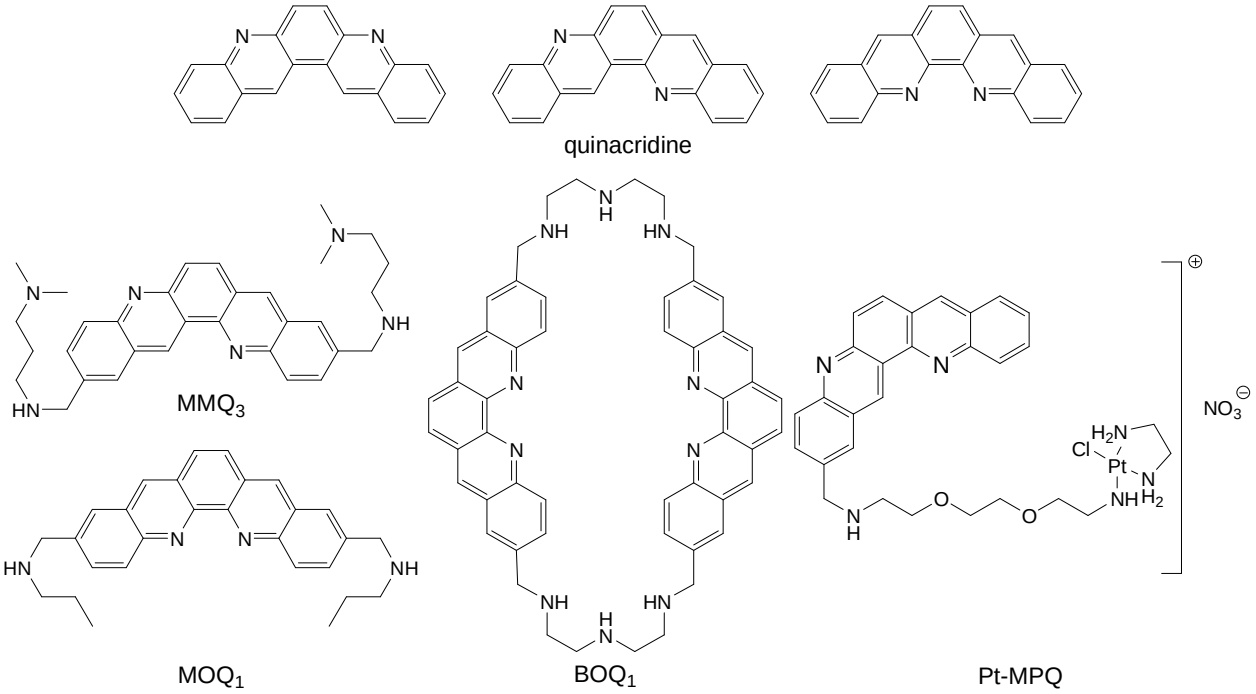
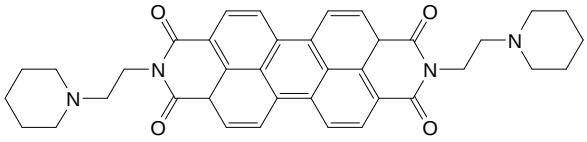


Figure 3.8: *Quinacridine G-quadruplex ligands*

Quinacridines (*Figure 3-8*) bind and stabilize human intramolecular quadruplex, as evidenced by changes in G-quadruplex melting temperatures (ΔT_m) that correlate quite well with telomerase inhibitory activities. The two most active quinacridies, MMQ_3 and its analog MOQ_1 have ΔT_m values of 19.7 and 12.5℃ and IC_{50} of 0.028 and 0.5 µM[90]. The connection of two quinacridine units by a polyamine linker in BOQ_1 may provide better selectivity for quadruplex structures since two planar quinacridines might stack on the G-tetrads, while the polyamine makes electrostatic interactions with the negatively charged phosphate groups of the DNA. BOQ_1 exhibits a ten fold higher binding affinity for intramolecular Q-quadruplexes than duplex DNA, whereas the monomeric substances are unable to discriminate. BOQ_1 exhibited a very strong stabilizing effect on the quadruplex (ΔT_m of +28℃) [91], as well as a submicromolar IC_{50} value for telomerase inhibition of 0.13 µM. In 2007 Teulade-

Fichoue *et al.* reported the first ligand which was shown to interact through noncovalent stacking interactions as well as covalent Pt binding: Pt-MPQ[92]. This interesting approach could lead to a new class of ligands directing *cis*-platinum compounds towards specific structures in the genome.

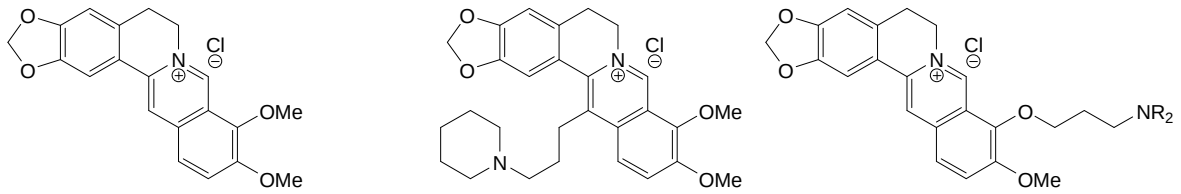


PIPER

Figure 3.9: *The perylene-based G-quadruplex ligand PIPER*

In 1998 Hurley *et al.* reported a series of substituted **perylene** G-quadruplex ligands having an extended aromatic core structure[93]. The most important example is "PIPER" (*Figure 3-9*). Upon *in situ* protonation of the piperidine substituents, PIPER showed a moderate telomerase inhibitory activity with an IC_{50} of around 20 µM. In NMR titration studies, binding stoichiometries of 1:2, 1:1 and 2:1 ligand/G-quadruplex were observed, depending on the sequence of the G4-DNA. PIPER is also capable of accelerating the assembly of G-quadruplex structures *in vitro*[94] and can induce the transition from duplex to G-quadruplex structures in the Pu27 sequence from the c-MYC promoter[95].

Berberine is an alkaloid isolated from Chinese herbs that has long been used as an antimicrobial agent. In 1999, berberine was reported to have an inhibitory effect on telomerase[96]. Subsequently, some substituted berberines were prepared and found to bind to G-quadruplexes, and to be capable of inducing G-quadruplex folding[97, 98].



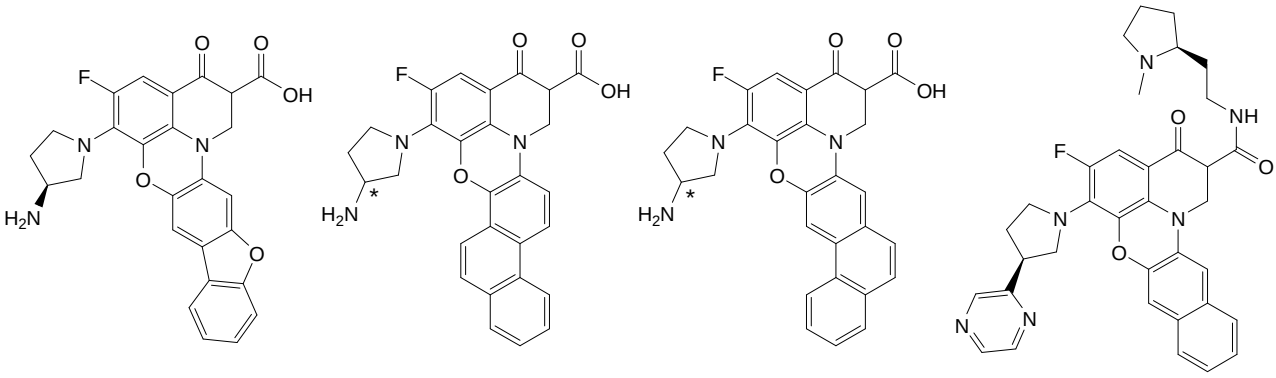
berberine 13-substituted berberie 9-substituted berberine

Figure 3.10: *Berberine-based G-quadruplex ligands*

Quinobenzoxazines are known for their unusual anti-bacterial activities[99]. Hurley's group designed the fluoroquinolone derivative QQ58 as the first example of a **quinoanthroxazine** (*Figure 3-11*)[100-102]. A NMR study revealed end-stacking of this compound onto the G-tetrads as the main binding mode of QQ58. Fluoroquinolones are also known to interact with topoisomerase II, as well as bacterial DNA gyrase[100,

[102-104]. The FQAs were found to have enhanced stacking interactions with G-quadruplexes and thus improved telomerase inhibition while maintaining the poisoning effect on topoisomerase II.

One compound from this class has been evaluated in human clinical trials. Quarfloxin CX-3543[105] was identified in optimization studies by Cylene Pharmaceuticals as having minimal topoisomerase II activity but enhanced G-quadruplex selectivity[100]. Since quarfloxin is accumulated in the nucleolus, its mechanism of action is thought to be selective disturbance of nucleolin DNA-complexes by binding to the G-quadruplexes of the ribosomal DNA. This has two proposed consequences: first, transcription of DNA by RNA polymerase I is inhibited, and secondly, nucleolin is transferred into the nucleoplasm where it might bind to the c-MYC G-quadruplex thereby turning off c-MYC transcription. The speculative story is nicely summarised by Stephen Neidle[106]. Quarfloxin is the first and only G-quadruplex ligand that has entered phase II clinical trials for treatment of neuroendocine/carcinoid tumours (ClinicalTrials.gov identifier: NCT00780663)[105].



QQ58 FQA-CS / FQA-CR FQA-TS / FQA-TR Quarfloxin (CX-3543)

Unfortunately it did not proceed to phase III due to poor bioavailability.

Figure 3.11: *Quinoanthroxazine G-quadruplex ligands*

A more recent group of selective G-quadruplex ligands is the **bistriazole** ligands, reported 2006 by Stephen Neidle *et al.*[107]. FRET analysis showed a moderate stabilizing effect of a G-quadruplex melting point with ΔT_m between +22 and 25°C at a concentration of 2 µM. Compared to BRACO-19 the bistriazoles show a small ΔT_m but better selectivity for G-quadruplex DNA. BRACO-19, increases the melting point of double stranded DNA by approximately 15°C, while all the bistriazoles had no effect on the melting of duplex DNA. The inhibition of telomerase according to TRAP assays is only modest with IC_{50} values ranging between 13 and 24 µM.

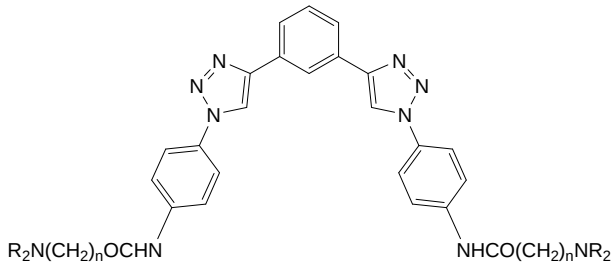


Figure 3.12: *Bistriazole G-quadruplex ligands*

Some triazine **bisquinoidinium** ligands (12459, 115405, *Figure 3-13*) were found to induce G-quadruplex formation in human telomeric DNA. They showed good telomerase inhibition with IC_{50} values in the nanomolar range. A newer series containing a pyridine in the center (compounds 307A, 360A, *Figure 3-13*) shows better selectivity for G-quadruplexes having a 33-150 fold preference compared to duplex DNA[108]. This represents about a ten fold improvement compared to the triazine compounds.

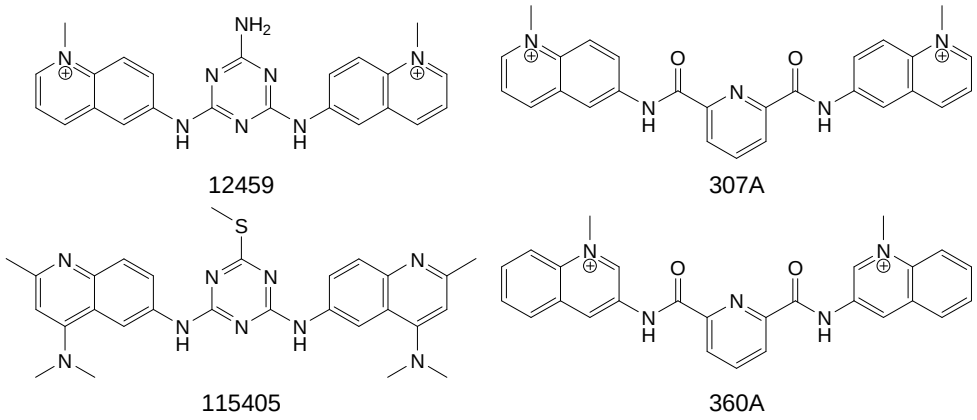


Figure 3.13: *Bisquinolidinium G-quadruplex ligands*

Bisquinolidiniums are likely to adopt a *syn-syn* conformation with amide hydrogen bonds to the central aromatic nitrogen resulting in a shape appropriate to end-stack on a G-tetrad. An enlargement of the central aromatic system to form a bipyridine or phenanthroline (*Figure 3-14*) was therefore conducted[109]. The strong stabilization properties of the resulting phenanthroline derivatives can be explained by enhanced stacking interactions with G-tetrads.

In addition to these families of derivatives, some other small molecules reported to bind to G-quadruplex DNA include: DODC, a carbocyanine dye specificially interacts with hairpin G-quadruplexes[110], quercetin[111], a natural product flavonoid and its glycosylated homolog rutin[112] (*Figure 3.15*).

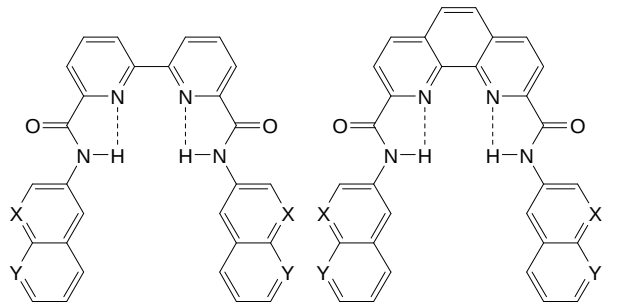


Figure 3.14: *Bisquinolidinium G-quadruplex ligands with an enlarged aromatic core*

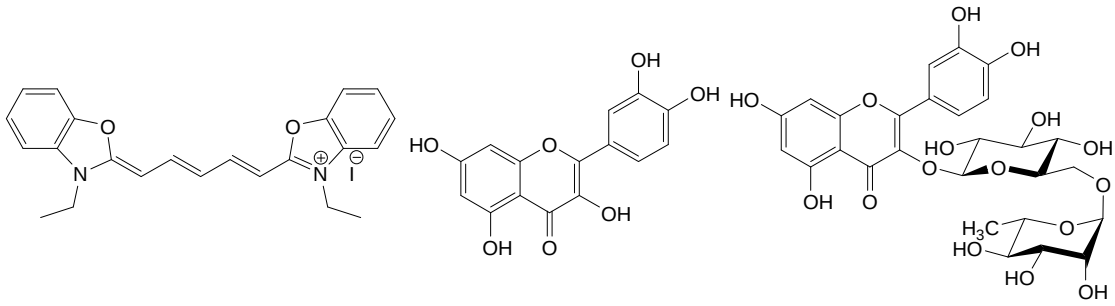


Figure 3.15: *Structures of DOCD, quercetin and its homolog rutin*

Metal complexes comprise a large group of G-quadruplex ligands. These include Ni(II)-salphen[113] and Cu-ttpy[114]. Ni(II)-salphen exhibits outstanding characteristics in terms of quadruplex stabilization: an impressive ΔT_m of 33℃ and a good selectivity for G-quadruplexes according to FRET-melting and SPR. Ni(II)-salphen is also a very potent telomerase inhibitor with an IC_{50} of 0.12 µM. The simple Cu(II)-terpyridine is less active, with a ΔT_m of 15℃ and a preference for G-quadruplex over dup lex of about 22.

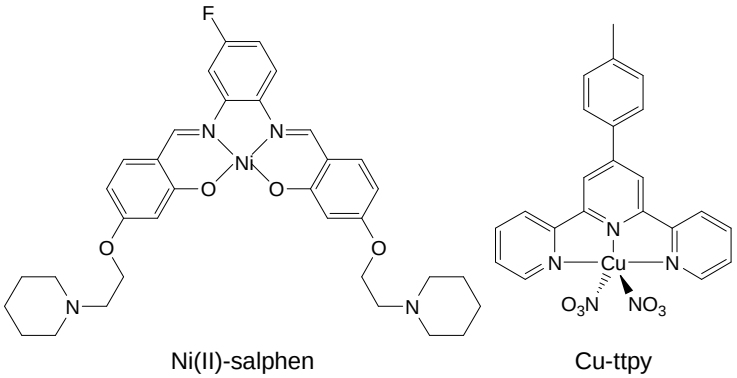


Figure 3.16: *Two metal complex G-quadruplex ligands: Ni(II)-salphen and Cu-ttpy*

3.2.2.2 Porphyrins and related macrocycles

Planar aromatic systems are ideal molecules for end-stacking onto G-tetrads. Porphyrins are well known as binding agents of duplex DNA. In 1998 Hurley *et al.* reported that the porphyrin TMPyP4 (tetra-(*N*-methyl-4-pyridyl)porphyrin, *Figure 3-17*) was a selective G-quadruplex ligand[115]. This molecule became one of the most intensely studied G-quadruplex ligands to date.

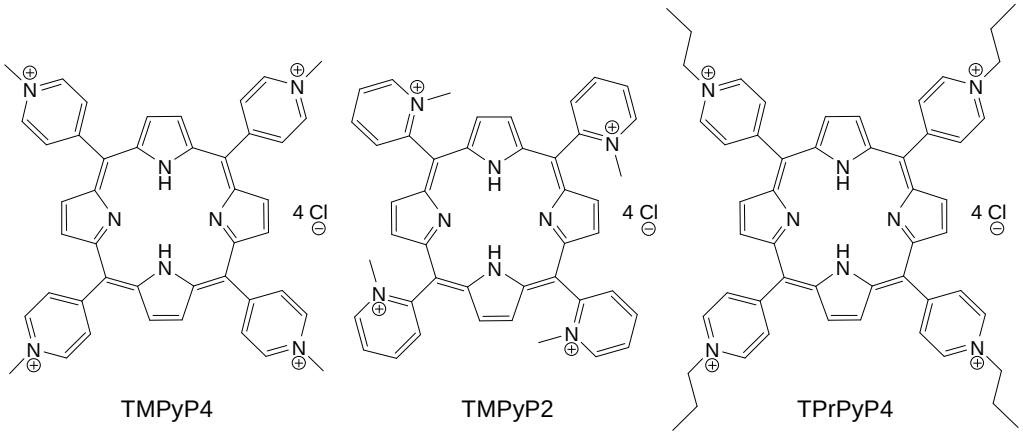
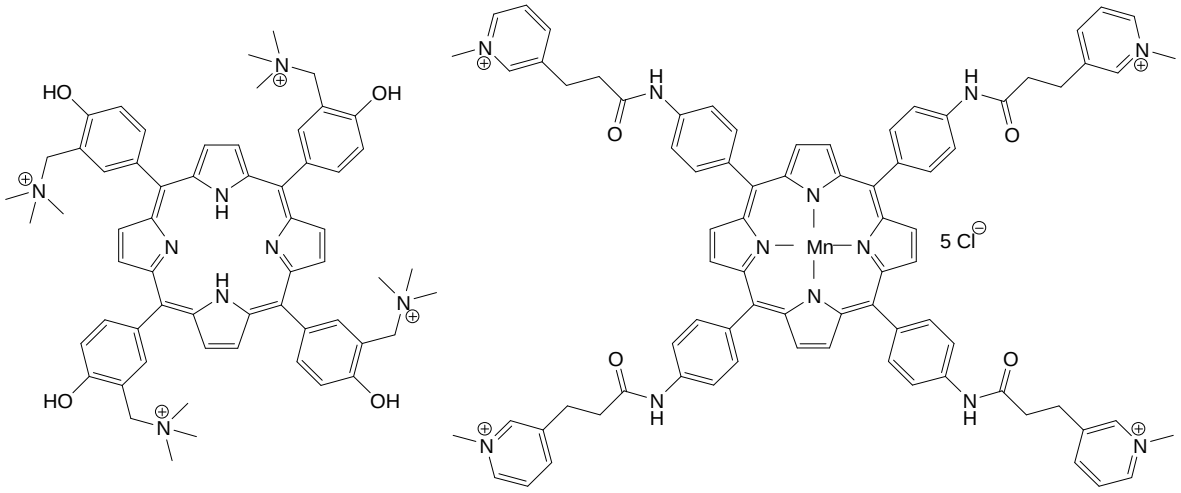


Figure 3.17: *The porphyrin-based G-quadruplex ligands TMPyP4, TMPyP2 and TPrPyP4*

Subsequent studies demonstrated that the binding stoichiometry of TMPyP4 is not clearly defined, and can range from 1-2[116-119], 4[119], even up to 5[120, 121] equivalents per quadruplex. These differences may be a consequence of non-selective DNA binding properties[62]. In fact, two distinctly different binding modes could be observed: 1) in a crystal structure TMPyP4 interacts with the bases of the connecting loops in a bimolecular 5'-d($TAG_3T_2AG_3$) telomeric DNA G-quadruplex[122] and 2) in an NMR structure, TMPyP4 stacks on the external G-tetrad planes of the Pu24I modified c-MYC G-quadruplex. The measured affinities for G-quadruplex and duplex DNA are K_d = 0.073 µM and 0.37 µM[123], indicating a only 5-fold preference for G-quadruplex. TMPyP4 stabilizes the G-quadruplex with a ΔT_m of +17°C, and it inhibits telomerase with an IC_{50} of 6.5 µM[115]. On the other hand, the isomer TMPyP2, with the *N*-methyl groups in the 2-position showed very weak activity[124, 125]. TMPyP4 was also tested in cells and showed a supression of the c-MYC[126] in the Ramos tumor cell line[127] and in the K562 leukemic cell line[128]. The BCL-2 promotor has also been found to interact with TMPyP4[129] and the ligand exhibits antitumor activity in retinoblastoma cell lines[130].

Structurally very similar to TMPyP4 is a tetra-propyl homolog TPrPyP4 (*Figure 3-17*). It is not very surprising, that the binding abilities of TPrPyP4 are similar to

TMPyP4: in a direct comparison with CD analysis both showed binding to a $(TGGGGT)_4$ in a ratio of 3:1: one ligand end-stacked and two lower affinity binding sites in the grooves[131].



TQMP Mn(III) porphyrin

Figure 3.18: *The porphyrin G-quadruplex ligands TQMP and a pentacationic manganese(III) complex*

A chemically reactive, TMPyP4 analog TQMP (*Figure 3-18*), exhibits similar non-covalent binding interactions, but it can be activated for covalent cross-linking upon photoexcitation. A Mn(III) porphyrin complex was reported to bind strongly to G-quadruplex DNA (K_d = 1 nM) with a 10'000 fold selectivity over duplex DNA and to inhibit efficiently telomerase (IC_{50} = 580 nM)[57]. A more recent publication reports 60-fold lower affinity and specificity for G-quadruplexes (K_d = 60 nM), but slightly higher telomerase inhibition activity (IC_{50} = 210 nM)[132].

Porphyrin-related oligopyrroles have also been synthesized and evaluated, (*Figure 3-19*). Corrole-type ligands, contracted by one methine group, exhibit good affinity (K_d = 0.46 μM)[127]. Examining positional effects in TMPyP4 related compounds revealed that *trans*-pyridinium groups are required for high affinity quadruplex interactions, *trans*-DMPyP4 has a K_d of 0.83 ± 0.10 μM, while *cis*-DMPyP4 has a K_d of 18.9 ± 2.80 μM[133].

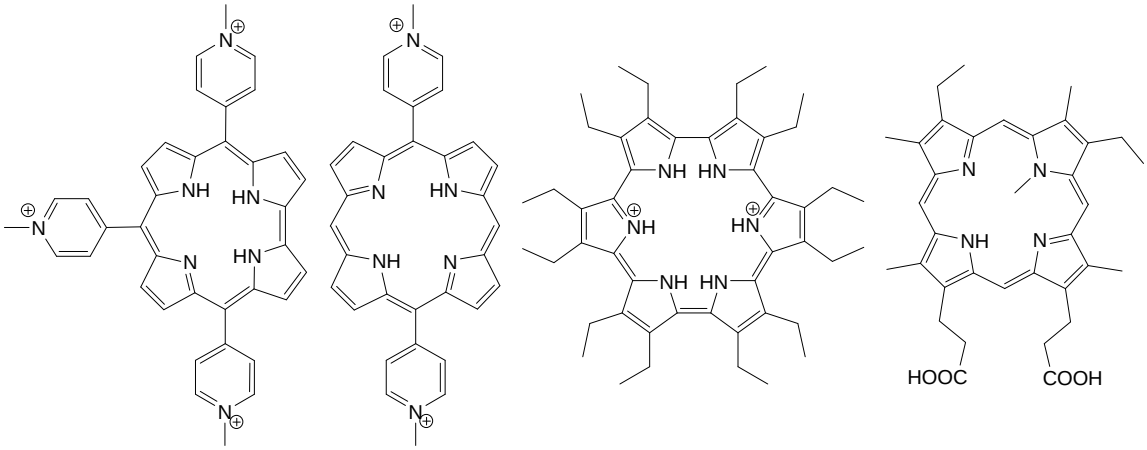


Figure 3.19: *Selection of different porphyrin-related G-quadruplex ligands*

A series of macrocyclic oligopyrroles was synthesised and analysed for quadruplex binding by Sessler and co-workers[134]. Dodecaethylcyclo[6]pyrrole was found by ESI mass spectrometry to have a higher affinity than the corresponding Cyclo[7]- and -[8]pyrrole macrocycles. This result was rationalized in terms of size complementarity with G-tetrads. While the vast majority of G-quadruplex ligands are cations, *N*-methyl mesoporphyrin (NMM) has negative charges on the side chains under physiological conditions. It was one of the very first G-quadruplex ligands ever reported, and it exhibits excellent G-quadruplex selectivity[62] but poor affinity[135-137].

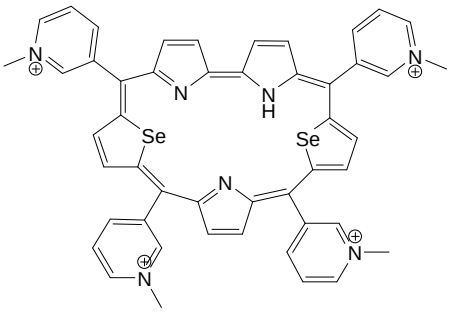


Figure 3.20: *The Se2SAP ligand*

Porphyrins do not have ideal shape complementarity with G-tetrads. Significant improvements can be obtained by expanding the macrocyclic core[138, 139]. Se2SAP was considered a breakthrough in the field of G-quadruplex ligands because it exhibits a better selectivity for quadruplex over duplex (around a 50-fold according to SPR studies). Se2SAP is also able to discriminate various topologies G-quadruplex-DNA. Unfortunately the synthesis of Se2SAP is very low yielding, a potential drawback for future exploration of its biological potential.

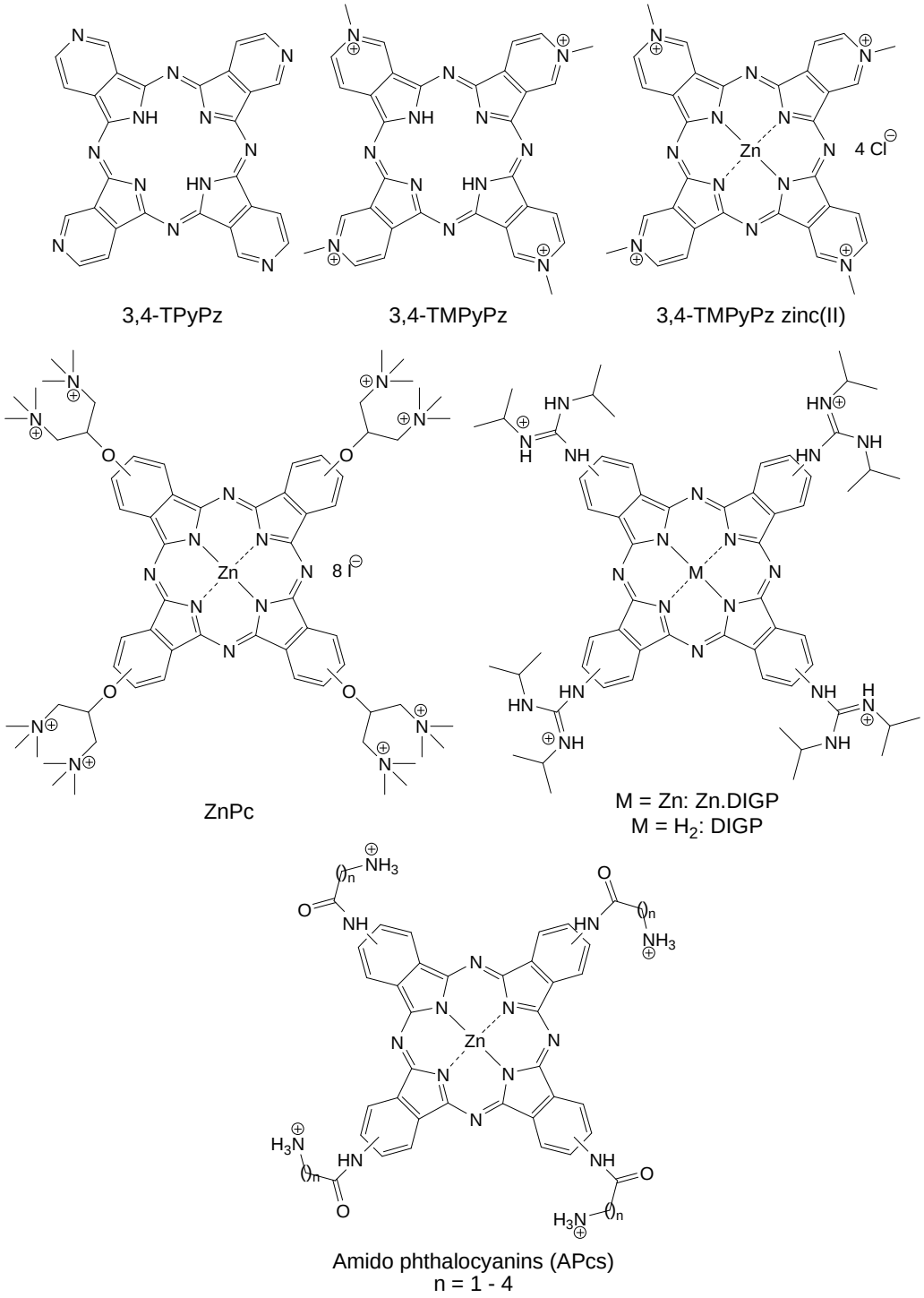


Figure 3.21: *Porphyrazine / phthalocyanine G-quadruplex ligands*

Phthalocyanines and their derivatives exhibit much better shape complementarity with G-tetrad DNA than do porphyrins. The methylated derivatives of non-symmetrical 3,4-tetrapyridinoporphyrazine (3,4-TPyPz), with four positive charges, induce the formation of antiparallel conformation of telomeric DNA and bind in a 1:1 ratio (K_d = 0.17 ± 0.02 μM for 3,4-TMPyPz and K_d = 0.40 ± 0.02 μM for 3,4-TMPyPz zinc(II)). The lower affinity of the Zn-containing ligand was explained by the higher

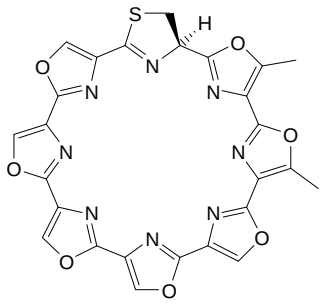
degree of self aggregation observed with this molecule. Phthalocyanines are generally potent G-quadruplex stabilizers, especially when coordinated to a Zn or Ni metal such as in the case of ZnPc, which has an affinity with a K_d = 0.12 μM and a telomerase IC_{50} = 0.23 μM[140, 141]. In 2009 Luedtke, Alzeer and co-workers published a new guanidinium-substituted phthalocyanine, Zn-DIGP, which has a K_d < 0.002 μM for c-MYC G-quadruplex DNA[56]. This is the strongest binding interaction between a G-quadruplex structure and a small molecule reported to date. Zn-DIGP is also outstanding in terms of specificity having a more than 5000-fold lower affinity for duplex than for quadruplex DNA. Another interesting feature of the Zn-DIGP is its fluorescence properties making it possible to demonstrate its cellular uptake in living NIH 3T3 and HeLa cells[142]. Unlike most other G-quadruplex ligands, Zn-DIGP is relatively non-toxic to cell cultures and is well tolerated upon intravenous injection into mice.

Phthalocyanines, porphyrins and perlyene derivatives exhibit a strong tendency to self-associate in aqueous solution. Whereas perlyene diimide exhibit increased binding specificity towards G-quadruplex structures upon aggregation, porphyrins and phthalocyanines loose their affinity upon aggregation. In 2010 Alzeer and Luedtke published their research on APcs (*Figure 3-21*)[143]. The self association of APcs is strongly pH dependent. Positive charges of protonated amino groups help prevent aggregation and the pK_a values of the substances depend on the terminal amino groups. With one methylene group separating the amine from the amide (n = 1), pH values ≥ 6.8 lead to aggregates incapable of binding to G-quadruplex DNA. Increasing the number of methylene units the pK_a is also increased, thereby decreasing aggregation and increasing G-quadruplex affinity.

3.2.2.3 Telomestatin and amide based macrocycles

In 2001, Shin-ya and his co-workers reported the identification and characterization of the natural product telomestatin. At the time, telomestatin was the most potent telomerase inhibitor reported to date with an IC_{50} of 5 nM[144]. Unfortunately, as already described in section 3.2.1, the initially measured IC_{50} was misleading because of PCR inhibition and has subsequently been revised to 0.9 – 1.2 μM[145] or 0.6 μM[64]. Notably, telomestatin exhibits an almost complete absence of affinity for duplex DNA[66], which is due to its neutral character and its cyclic shape.

Telomestatin was reported to have at least a 70-fold preference for G-quadruplex over duplex[146].



1
telomestatin

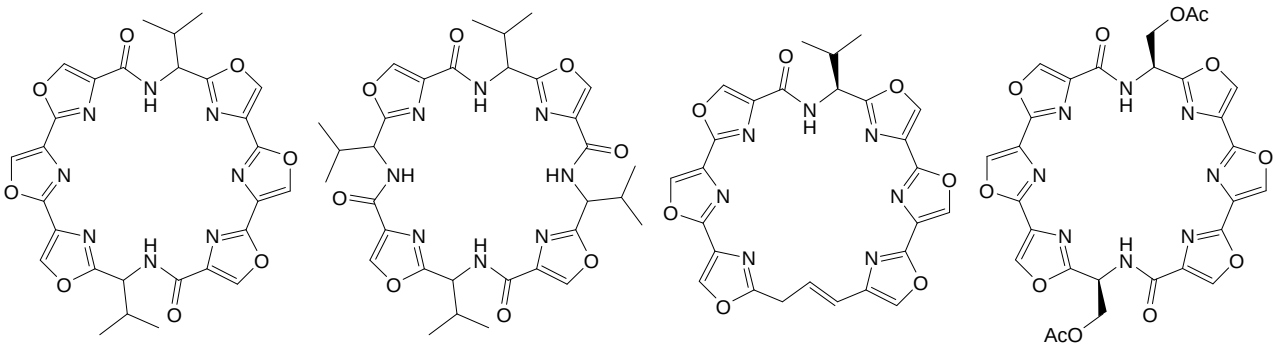
Figure 3.22: *Telomestatin: the revolutionary quadruplex ligand*

Telomestatin (**1**) exhibits a wide variety of biological effects including: (1) inhibition of cell growth in cancer cells but not in normal fibroblasts, (2) no significant telomere attrition in either cancer or non-cancer cells under short-term conditions, (3) reduction of 3' telomeric overhangs, (4) induction of POT1 dissociation from telomeres, (5) induction of anaphase bridge formation and apoptosis[147]. The reasons behind these biological activities is probably the high affinity of telomestatin for intramolecular G-quadruplex structures, having a ΔT_m = + 24°C. Telomestatin changes the normal conformational preferences of DNA[148]. It causes telomere uncapping and release of telomeric proteins (POT1) and leads to G-overhang degradation which is associated with DNA damage response[23, 148]. Telomestatin was also found to have an effect on cancer cells that lack telomerase overexpression known as ALT (Alternative Lengthening of Telomeres). In ALT cells, telomestatin also induces G-overhang degradation and provokes a massive DNA damage response[148]. Interestingly, telomestatin does not disturb the replication process of telomeres, even after extended treatments[149]. These results suggest that the proposed models for telomerase inhibition and telomere shortening may not be viable.

One major drawback of telomestatin is that it is difficult to obtain. Despite many unsuccessful attempts[150-156], there is only one total synthesis described in literature[157]. This route is clearly incompatible with large scale production. The bacterial production of the molecule is also quite low due to inefficient fermentation. This approach is also incompatible with the production of derivatives.

Given the extraordinary G-quadruplex specificity and biological activities of telomestatin, some related analogs were synthesised and characterised. One of the first examples was hexaoxazole HXDV, reported 2006 by Rice and co-workers[158]

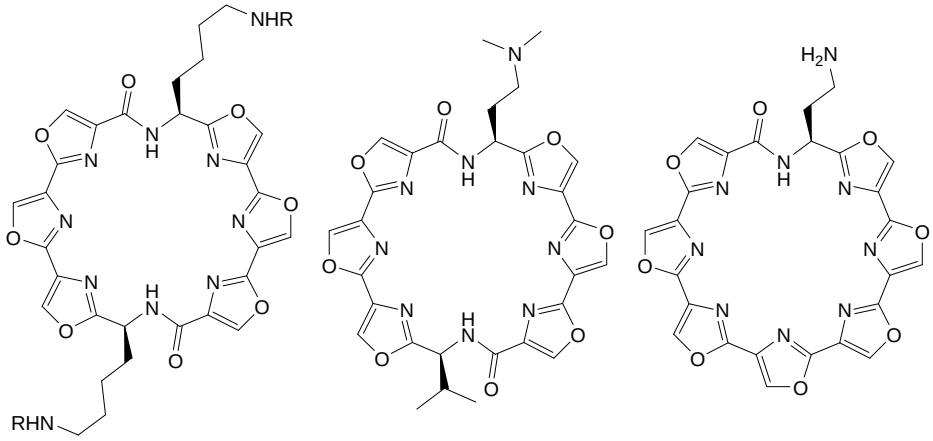
(*Figure 3-23*). This macrocycle binds selectively to quadruplexes by stacking onto the terminal G-tetrads, and it does not bind to duplex or triplex DNA[159, 160].



HXDV **2** **3** bistrioxazole acetate

Figure 3.23: *HXDV and related telomestatin analogs*

Rice compared HXDV with the tetra-isopropyl substituted compound **2** and found that HXDV stabilized a telomeric G-quadruplex by ΔT_m = + 17.5℃, while **2** did not have any stabilizing effect[158]. While EC_{50} values for HXDV ranged from 0.3 – 8 μM, a related derivative **3** was found to be somewhat more toxic having EC_{50} between 0.025 – 3.3 μM[161]. Unfortunately, it is not reported if the increase in cytotoxicity is cancer cell specific or not. The bistrioxazole acetate was also tested for telomerase inhibition and revealed an IC_{50} of 2 μM[162].

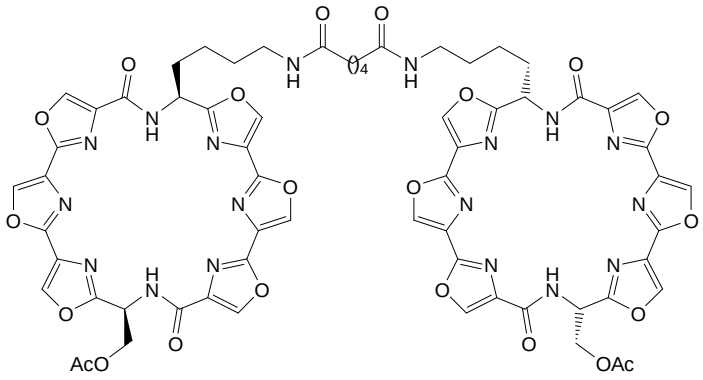


4 **5** **6**

Figure 3.24: *Telomestatin analogs with cationic side chains*

While porphyrin-based G-quadruplex ligands typically have a planar core with positively charged side chains, telomestatin-like molecules are typically neutral and therefore more drug-like, but exhibit very limited water solubility. Cationic side chains could help to improve water solubility while, at the same time, enhancing the affinity and selectivity between different types of G-quadruplex topologies. A series of cationic derivatives was therefore synthesised and evaluated.

Diaminobutylhexaoxazole **4** exhibited a superior ability to stabilize telomeric DNA as compared to HXDV and related analogs containing only one amino group[163]. It also exhibited a preference for the antiparallel telomeric G-quadruplex structure over the parallel one[164]. The effect of the side chain variation was recently demonstrated by Rice and co-workers[165]. The [(dimethyl)amino]ethyl-isopropylhexaoxazole **5** displayed high potency with respect to G-quadruplex stabilization, reaching ΔT_m values of +21.5℃ and + 34.5℃ for hTel and AurA DN A, as well as potent cytotoxic activities. **5** demonstrated *in vivo* a potential as anti-cancer agent: in mice containing human tumor xenografts it reached a T/C-value (average tumor volume as compared to control group) of 21.6%. Heptaoxazole **6**[166], was also found to stabilize antiparallel telomeric G-quadruplexes. The free amino group which can be protonated under physiologic conditions, plays a significant role in the binding interaction since a corresponding *N*-acetylated derivative has revealed three-fold lower potency. **6** has cytotoxic activity in telomerase-positive HeLa cells (IC_{50} = 0.0022 μM) whereas telomerase-negative Saos-2 cells are unaffected.



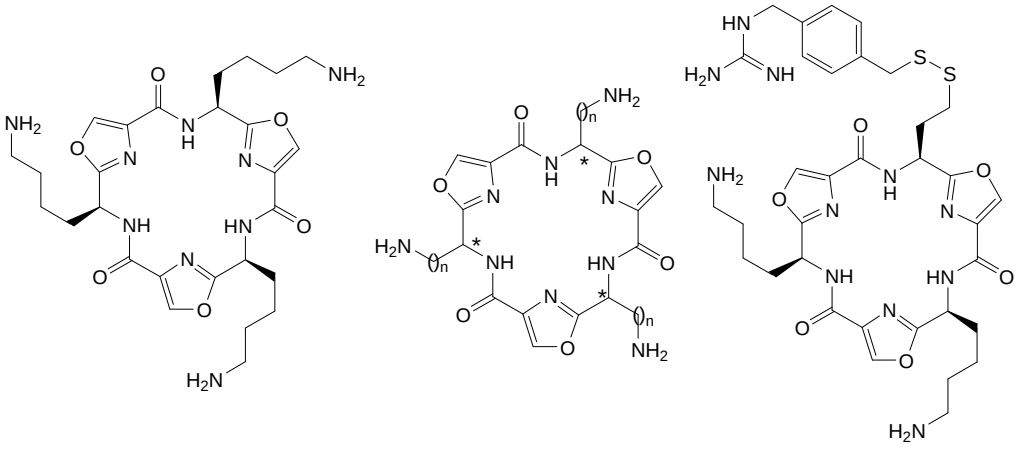
7

Figure 3.25: *A dimeric hexaoxazole G-quadruplex ligand*

A clever idea was reported recently by Shin-ya *et al.*[167] connecting two hexaoxazoles by linkers in different length. A ten-fold higher selectivity for telomeric DNA as compared to a mutated telomeric sequence was reported for dimer **7** versus the corresponding monomer. The two connected hexaoxazoles are expected to interact as terminal caps on the same quadruplex, whereas the monomer interacts with a 2:1 binding mode similar to telomestatin and HXDV.

A series of trisoxazole macrocyclic ligands shown in *Figure 3-26* was shown to interact selectively with the parallel versus antiparallel telomeric G-quadruplex structure[168]. Having four CH_2 units between the macrocycle and amine was found to be much better than having just one. A series of four diastereoisomers was tested

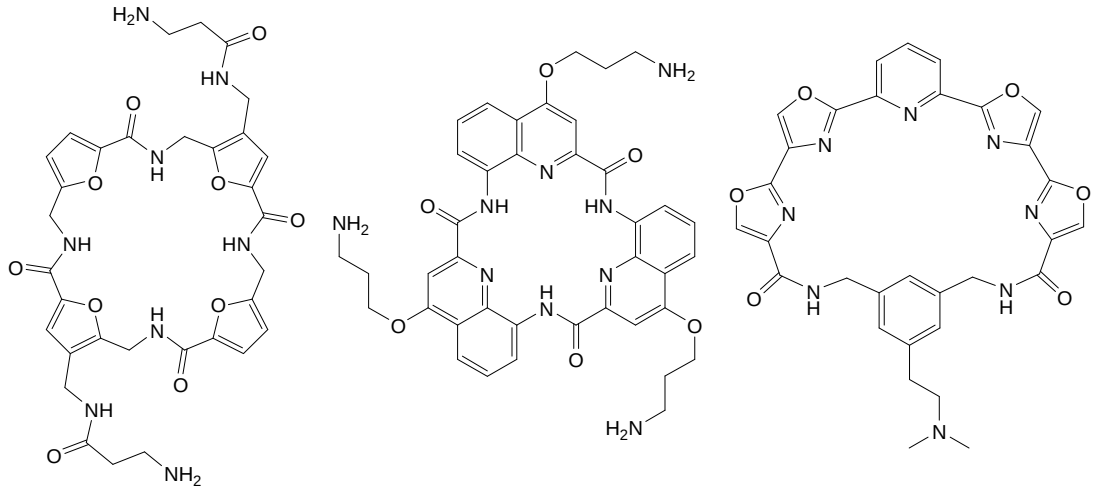
and found to be very similar. The (*S*,*S*,*S*) compound exhibited a G-quadruplex stabilization of ΔT_m = + 10.8°C as compared to the (*R*,*R*,*R*)-compound with ΔT_m = + 8.7°C. These compounds also exhibited similar affin ity (K_d(*S*,*S*,*S*) = 5 ± 2 µM and K_d(*R*,*R*,*R*) = 4 ± 2 µM).



8

Figure 3.26: *Substituted trioxazole G-quadruplex ligands*

Upon screening a dynamic combinatorial library with G-quadruplex templates, guanidinium-containing trisoxazole **8** was identified to be a preferred binding partner for both antiparallel and parallel G-quadruplexes[169]. The related 24-membered macrocycle **9** (*Figure 3-27*) containing four furan units[170] proved to be selective for telomeric DNA with K_d value of 3.1 µM, but exhibited no telomerase inhibition activities. The 18-membered quinoline-based macrocycle **10** was shown to stabilize parallel (c-Kit) and antiparallel (h-telo) DNA quadruplexes with ΔT_m values of + 21.4°C and + 33.8°C respectively [171]. This ligand has an even better stabilizing effect than telomestatin itself, which has "only" ΔT_m of + 20.4°C and + 30.3°C [172]. More recently, the pyridine- and oxazole-containing 24-membered macrocycle **11** was published by Rice and co-workers[173]. It also exhibited high stabilization of DNA and RNA quadruplex structures, having a ΔT_m = + 20.5°C (in case of h-telo DNA) or ΔT_m = + 37.0°C (in case of AurA RNA). This compound als o exhibited anticancer activities in mice having human breast cancer xenograft, with no reported toxic effects.



9 **10** **11**

Figure 3.27: *24- and 18-membered macrocyclic G-quadruplex ligands*

3.3 Synthetic strategies for telomestatin type ligands

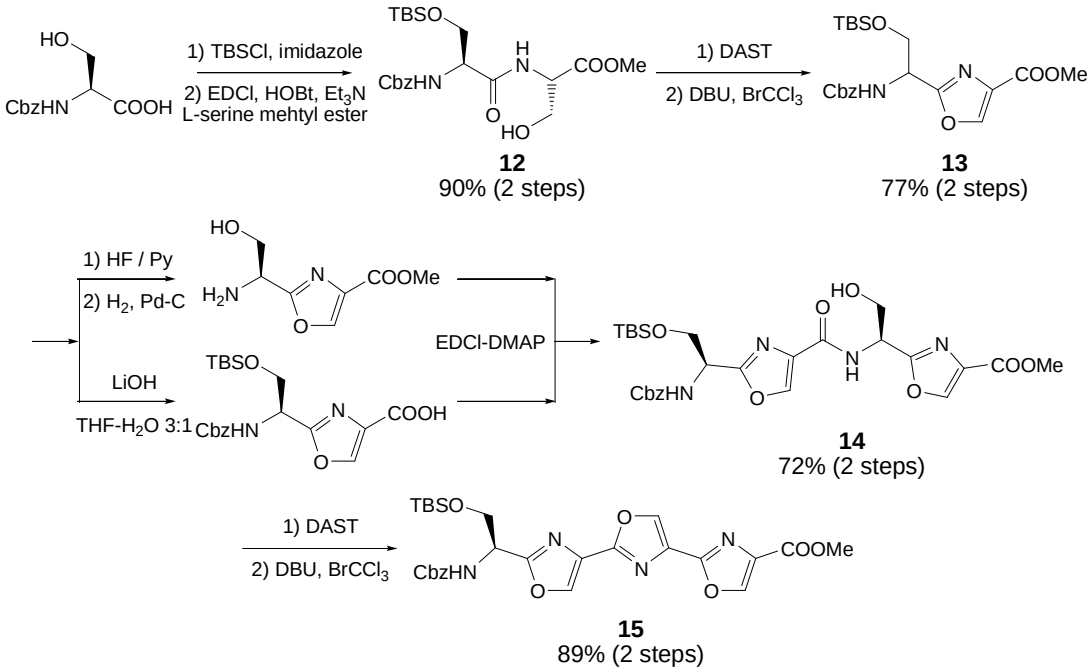
What makes the synthesis of telomestatin and related compounds difficult and how are these problems solved? In the following three sections the synthetic paths to these compounds are described.

3.3.1 Synthesis of telomestatin-related hexaoxazoles

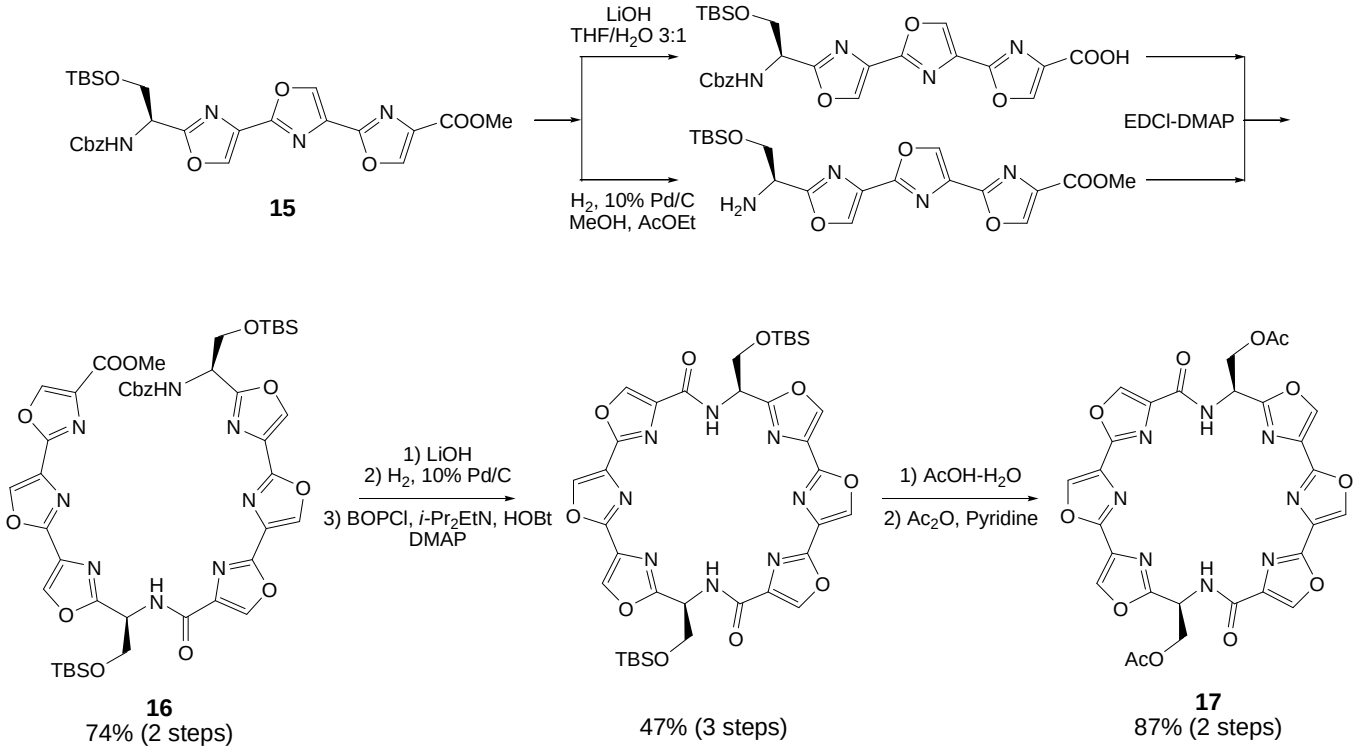
A series of hexaoxazoles was synthesised by Shin-ya and co-workers using a convergent synthesis[162]. The compound can be built from eight serine amino acids applying different protecting groups. The amino acid couplings were performed using 1-[3-(diethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCl). The resulting diserine **12** (*Scheme 3.1*) was converted to an oxazole amino acid using a two step reaction with the fluorinating agent DAST followed by DBU / $BrCCl_3$. The resulting compound **13** was deprotected and dimerised to give **14**. Following serine deprotection, a third oxazole unit was generated using DAST / DBU, $BrCCl_3$ to give **15**, representing half of the target molecule. Trioxazole **15** was again dimerized giving the linear hexaoxazole **16** (*Scheme 3.2*). After deprotection macrolactamization was performed under high dilution (1 mM) using *N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl) and diisopropylethylamine in a mixture of CH_2Cl_2 and DMF. A final deprotection provides the opportunity to insert different side chains, for example an acetyl group, to give compound **17**. Shin-ya claims that these compounds were designed on the structural basis of telomestatin in search for a possibility to increase the binding interaction by insertion of side chains. Around the

same time, Rice *et al.* published HXDV (*Figure 3-23*) following more or less the same synthetic strategy[158]. Two years later, Shin-ya described analogous macrocycles having amine-containing side chains instead of the alcohols[164].

Scheme 3.1: *Synthesis of the trisoxazole*[162]

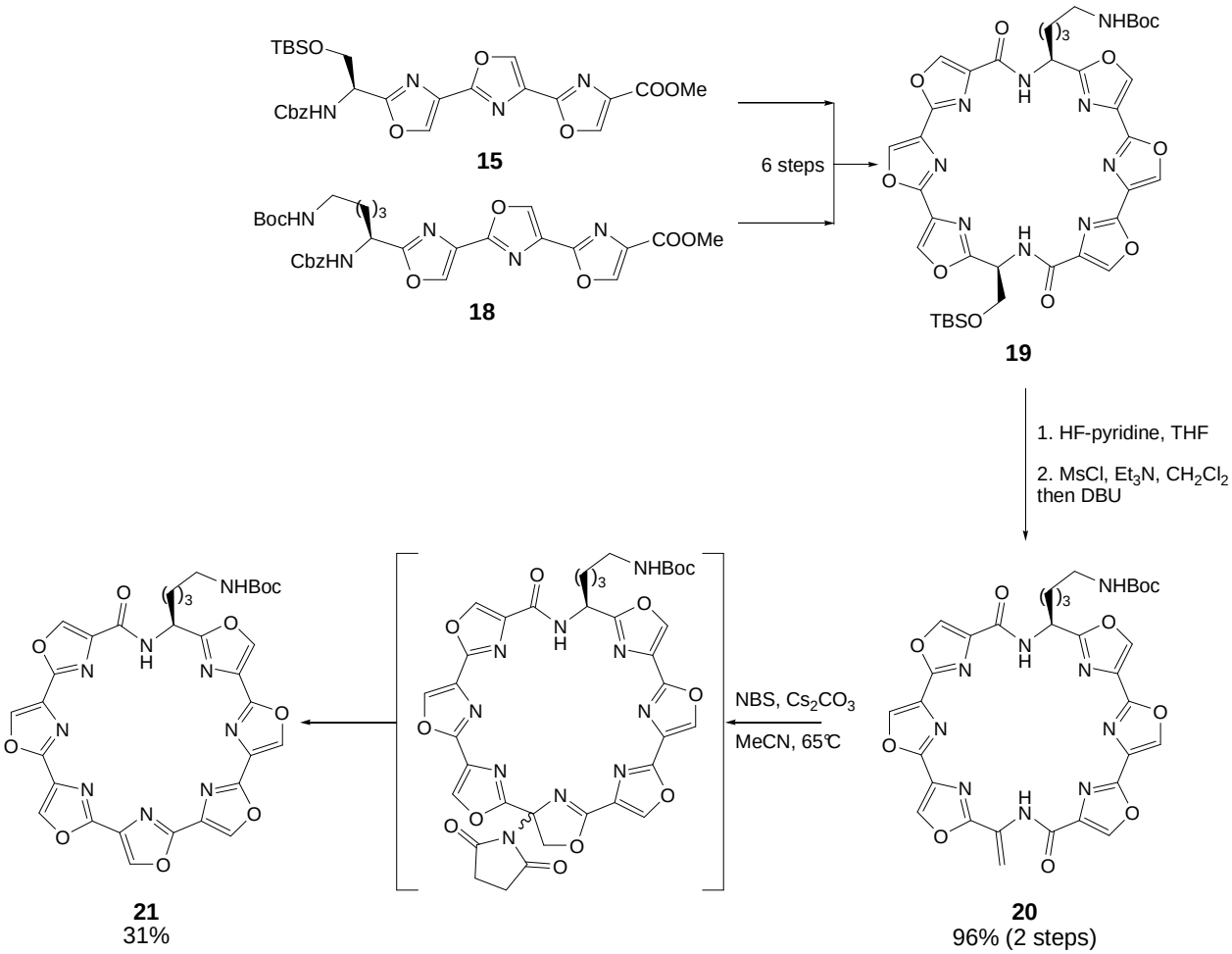


Scheme 3.2: *Synthesis of hexaoxazoles*[162]



3.3.2 Synthesis of telomestatin-related heptaoxazoles

Scheme 3.3: *Synthesis of heptaoxazoles*[166]



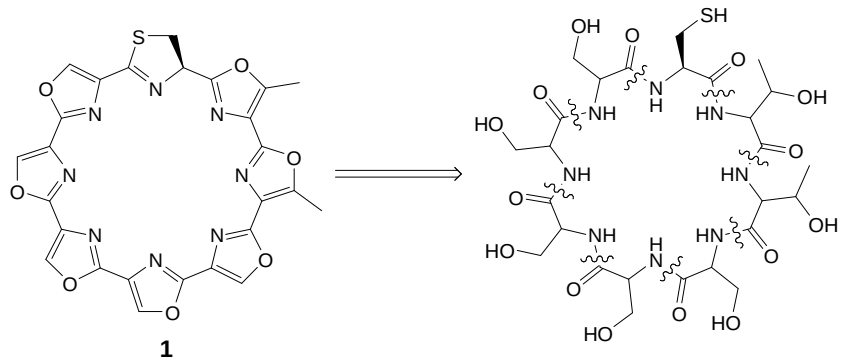
Following his total synthesis of telomestatin and related hexaoxazoles, Shin-ya and co-workers moved to the synthesis of heptaoxazoles[166]. The reported convergent synthesis of heptaoxazoles is similar to that used for the hexaoxazoles. The key difference is the use of two different trioxazoles resulting in orthogonally protected side chains on the macrocycle. As one of the trioxazoles the serine-derived TBS-protected alcohol **15** was used. The other trioxazole **18** was synthesised, having a Boc-protected aminobutyl group derived from L-lysine[162, 164] (*Scheme 3.3*). In six steps the two halfs were transformed into macrocycle **19**. Having now two orthogonally protected side chains, the alcohol was deprotected and eliminated using mesylation and DBU to furnish the enamide **20**. This could be reacted with *N*-bromosuccinimide (NBS) and Cs_2CO_3 in MeCN at 65℃ to generate the heptaoxazole **21**. This unconventional path was likely chosen since the typical reagents like DAST probably did not work in the case of the hexaoxazole. It is thought that the strained

structure of the β-hydroxyamide moiety is not compatible with this reaction[150, 155, 157-159, 163].

3.3.3 Total synthesis of telomestatin

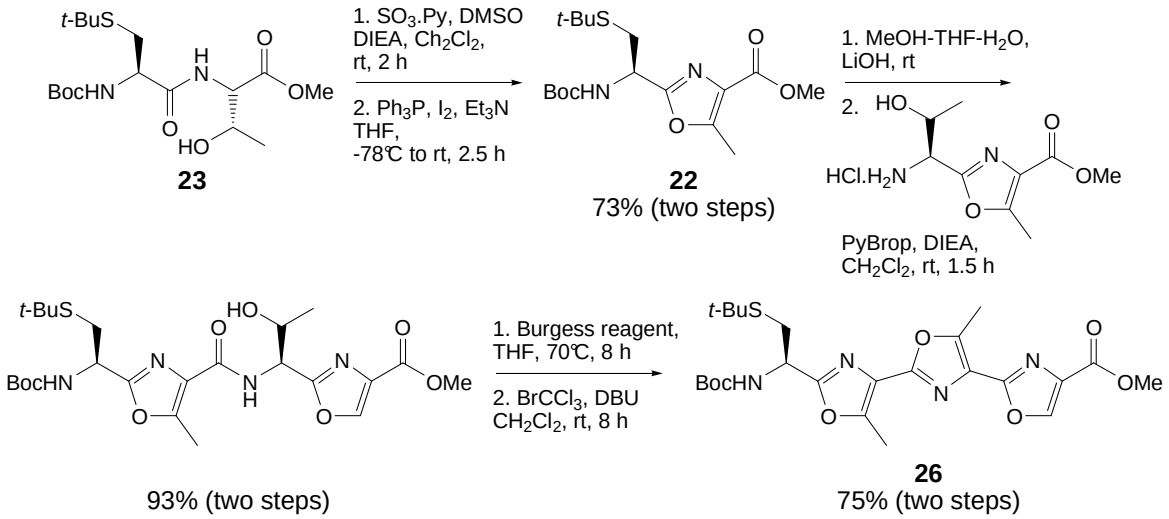
The natural product telomestatin is more complicated than the simplified analogs described so far. The symmetry of the compound is much lower, having one thiazoline ring and two methyl oxazole substituents. Biosynthetically it is most likely synthesised from the three amino acids serine, threonine and cystein as shown in *Scheme 3.4*[174].

Scheme 3.4: *Retrosynthesis to the most probable building blocks for biosynthesis of telomestatin*



1
Telomestatin

Scheme 3.5: *Synthesis of the methyl substituted trioxazole as building block for telomestatin*



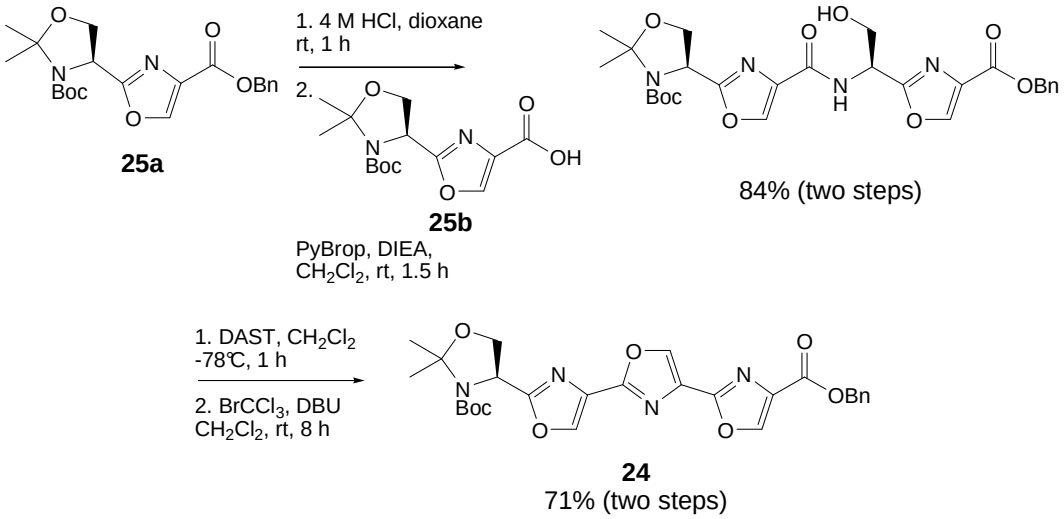
To date, only a single total synthesis of telomestatin has been reported[157]. The previously described convergent synthesis (*Scheme 3.1, 3.2, 3.3*) can also be applied in the case of telomestatin (*Scheme 3.5, 3.6*). The lower symmetry makes

the fragment synthesis somewhat more difficult. The formation of the thiazoline, the only non-oxidized, and potentially labile ring, is the last to be closed.

The synthesis of building blocks for telomestatin was performed in analogy to the previously reported trioxazoles: the amino acids were coupled and the side chains cyclized and oxidized. For the formation of 5-methyl-1,3-oxazole **22** the secondary alcohol **23** was first oxidized to the ketone with SO_3-pyridine and DMSO (Parikh-Doering oxidation) and immediately cyclodehydrated using Ph_3P and I_2 in presence of Et_3N (*Scheme 3.5*). Shin-ya does not give any reasons for this unusual strategy. Again it could be due to the failure of the DAST and DBU, $BrCCl_3$ procedure.

The synthesis of trioxazole **24** (*Scheme 3.6*) differs from the other examples only in the protection of the *N*-terminal amino and hydroxy group as acetonides forming an oxazolidine. Two such units, **25a** and **25b** were fused. The rest of the synthesis was performed in analogy to building block **26**.

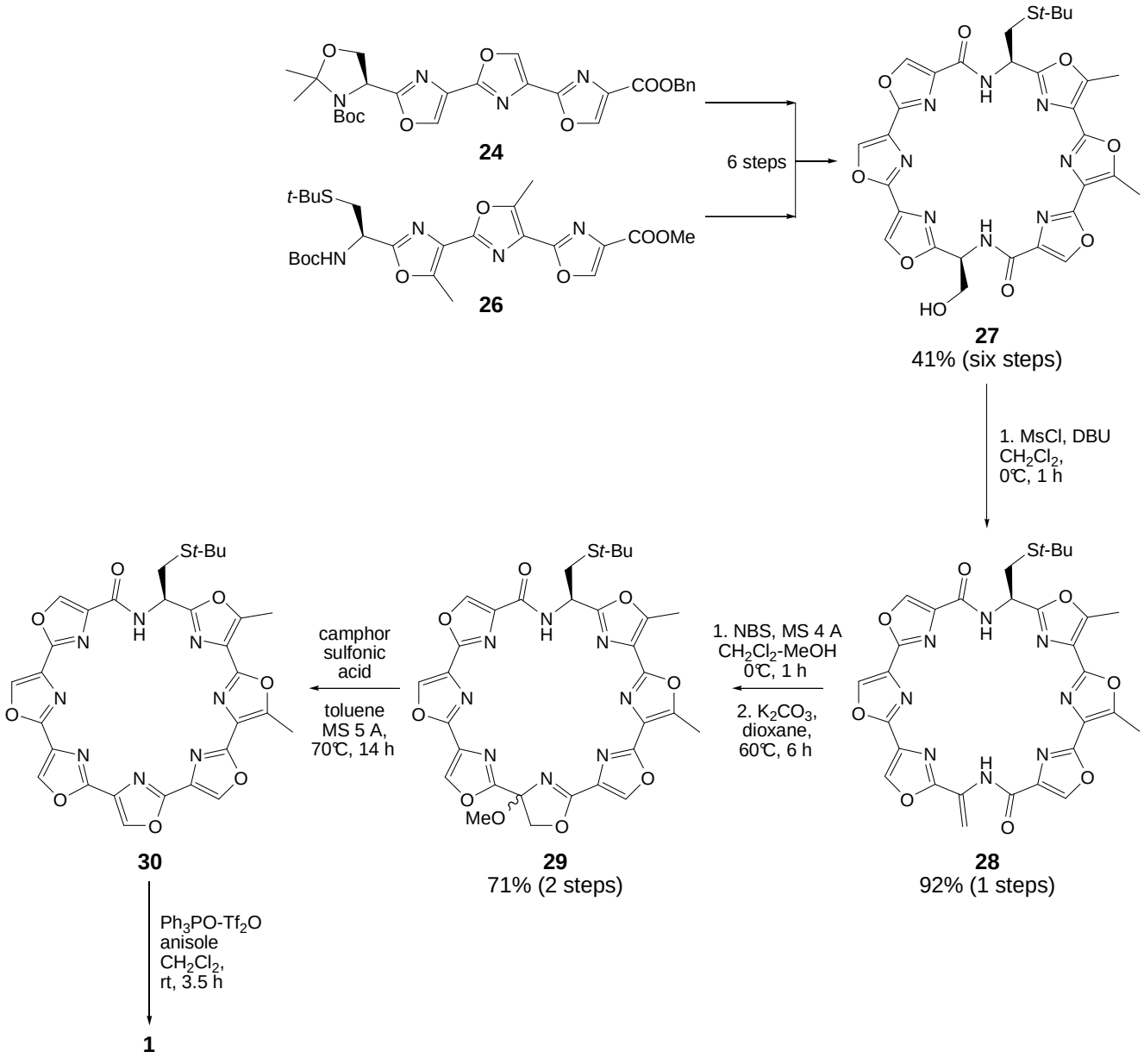
Scheme 3.6: *Synthesis of the second trioxazole building block for telomestatin*



The fusion of trioxazoles **24** and **26** (*Scheme 3.7*) was conducted analogously to the previously described syntheses. The macrolactamization was done under high dilution (3 mM) in a mixture of CH_2Cl_2 and DMF at rt over 3 days using DPPA[175] – HOBt and DIEA in the presence of DMAP as coupling reagents in an overall yield of 48%. The subsequent diamide dehydration to the oxazoline did not work with DAST or Burgess reagent, resulting only in elimination. Oxidation of the primary alcohol **27** followed by cyclodehydration also failed. The enamide **28** could be reached in 92% by mesylation of the alcohol with DBU. According to an earlier report by Shin-ya[150], the enamide **28** could be used to form an α-methoxy-β-bromo cyclic peptide in 90%.

For this process, it is crucial to add molecular sieves (4 Å), otherwise the sulphide moiety is partially oxidized leading to decomposition. The α-methoxy-β-bromo cyclic peptide was subsequently cyclized to afford the 4-methoxyoxazoline **29** using K_2CO_3 at 60℃. The methoxy group could be selectively eli minated in toluene at 70℃ with camphorsulfonic acid and molecular sieves (5 Å) to give the heptaoxazole **30**. Again, molecular sieves were important, this time to prevent the oxazoline from being hydrolyzed back to the alcohol. The thiazoline was formed using a modified method described by Kelly[176], using Ph_3PO, Tf_2O and anisole in CH_2Cl_2 at room temperature. The yield of this last synthetic step was only 20% due to the limited solubility of telomestatin during purification.

Scheme 3.7: *Total synthesis of telomestatin by Takahashi*[157]



The spectroscopic data from this product were in agreement with those from the isolated natural product. The total synthesis could therefore also confirm the stereochemistry of telomestatin, the only stereocenter having an *R*-configuration, consistent with a natural Cys precursor (*Scheme 3.7*).

3.4 Current problems and goals

After ten years of intensive research in the field of G-quadruplex ligands, there is still no compound that has reached drug status. Clinical research into telomestatin ended at the pre-clinical stage due to toxicity in animal models.

Unlike many G-quadruplex ligands, telomestatin and its derivatives are "drug-like" (according to Lipinski's rules, telomestatin has a molecular weight of 582.504 g/mol, no H-bond donors, 16 H-bond acceptors, no rotatable bonds). Telomestatin is an attractive scaffold for further diversification. New telomestatin-like G-quadruplex ligands are therefore needed to address the following goals:

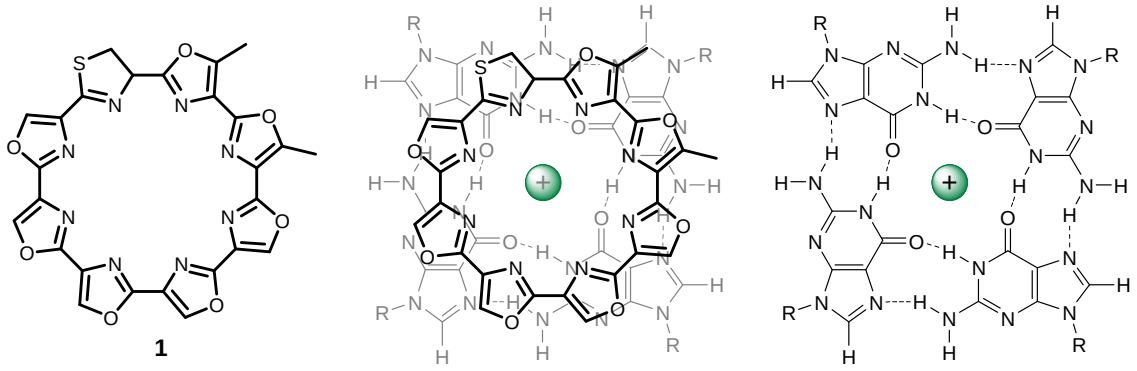
- To further improve the binding affinity towards G-quadruplexes and selectivity between different G-quadruplex sequences;
- To improve the solubility of the molecule;
- To achieve good pharmacokinetics;
- To reduce the toxicity of the compounds.

We will attempt to increase G-quadruplex binding affinity by increasing the planarity of molecules having telomestatin-like core structures. Selectivity between G-quadruplexes will be achieved by placing substituents on the core to interact with the DNA back bone or groove regions. Proper choice of substituents will also improve the solubility properties and modulate the pharmacokinetics and toxicity of derivatives *in vivo*.

4. Design of New Telomestatin Analogs

4.1 Structural features of telomestatin

The natural product telomestatin exhibits excellent G-quadruplex affinity and specificity[146, 177]. Telomestatin stacks onto the terminal G-tetrads of intramolecular G-quadruplexes. A two to one binding stoichiometry was determined by Valérie Gabelica *et al.* by electronspray mass spectrometry[58].



1

Figure 4.1: *Telomestatin (left), the G-tetrad (right) and an overlay of the two structural representations, drawn in accordance with* in silico *calculations described by Maiti*[178]

There are no high resolution NMR or X-ray structures of any telomestatin-G-quadruplex complexes available to date. Only two *in silico* investigations have been reported, that use known G-quadruplex structures and manual docking of telomestatin[29, 177, 178]. These modelling studies suggest that telomestatin has approximately the right size to fit onto the G-tetrads of G-quadruplexes. It has been reasoned that the relative flatness of the macrocycle facilitates good stacking interactions between the oxazole and the guanine aromatic heterocycles. In this respect, telomestatin has good, but not optimal shape. Due to the presence of a single thiazoline unit, telomestatin is not a planar molecule (*Figure 4.2*). This should limit the potential stacking interactions between telomestatin and planar G-tetrads. While the exact function of telomestatin in context of *Streptomyces anulatus* 3533-SV4 is unknown, it is a reasonable question to ask why the sulphur-containing ring is not oxidized like the other rings. Potential limitations to this oxidation reaction include ring-strain (discussed in section 4.2) and solubility. Limited solubility properties have been reported in context of the total synthesis of telomestatin by Shin-ya *et al.*[157]. The final step of the synthesis yields only 20% due to purification difficulties

associated with poor solubility during both normal and reversed phase chromatography.

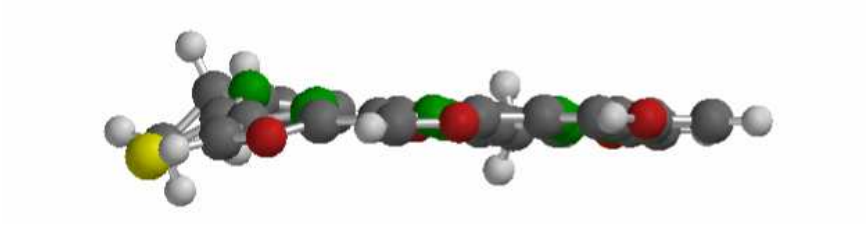


Figure 4.2: *Side view on telomestatin (Density Functional / SVWN/DN**-optimized structure)*

Another potential reason for the lack of high resolution structural data of telomestatin-quadruplex complexes is the lack of symmetry. The two methyl substituents and the thiazoline reduce the symmetry of telomestatin to C_1. Combined with the pseudo C_4 symmetry of G-tetrads, defined positioning of telomestatin is difficult and probably results in multiple stacking orientations between telomestatin and G-tetrads. These properties complicate the structural elucidation of the complexes in terms of X-ray analysis and NMR-based structural studies. Increasing the symmetry of the ligand, to C_4 for example, would be an advantageous feature of new telomestatin analogs.

4.2 Macrocyclic ring-strain of telomestatin

DFT-optimized structures of telomestatin suggest a shape that is nearly planar over 2/3 of the molecule (*Figure 4.2* and *4.3*). A linear analog was generated *in silico* by cutting a single bond in the macrocycle and re-optimizing the structural energy. The resulting structure shows a strong tendency to open, revealing the presence of macrocyclic ring-strain in telomestatin (*Figure 4.3* on the right). Similar virtual experiments using a telomestatin analog with 10 oxazole units suggest the addition of two oxazoles to telomestatin would relax all ring-strain. This change also alters the size of the molecule and docking studies suggest that [$0._{10}$](2,4)1,3-oxazolophan (**31**) would not necessarily have good shape complementarity with G-tetrads (*Figure 4.4*). A more quantitative virtual experiment to estimate ring-strain has been conducted by calculating the number of five-membered rings needed to form a perfectly circular macrocycle. In a geometry-optimized 2,4-dimethyl-1,3-oxazole, the angle ϕ is defined as the two perpendicular bisectors of the bonds between each methyl group and

oxazole (*Figure 4.5*). The calculated angle ϕ is 35.14°, so 10.2 oxazoles are expected to fill a circle of 360°. These estimates are substantiated by angles derived from crystallographic data (*Figure 4.6*).

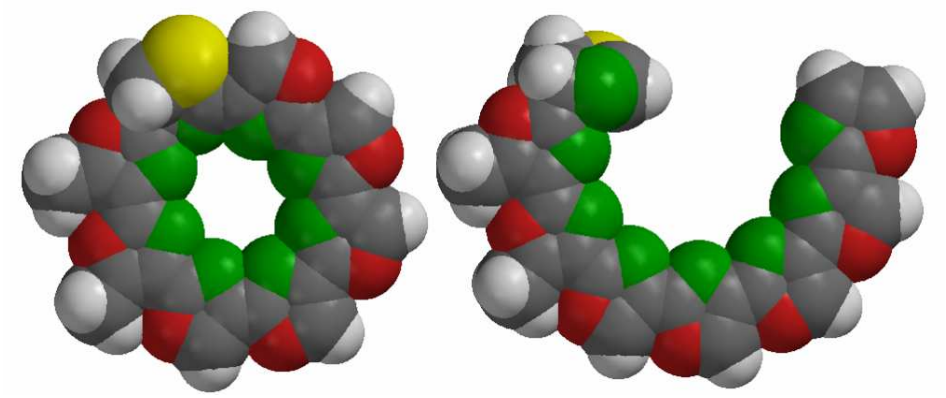
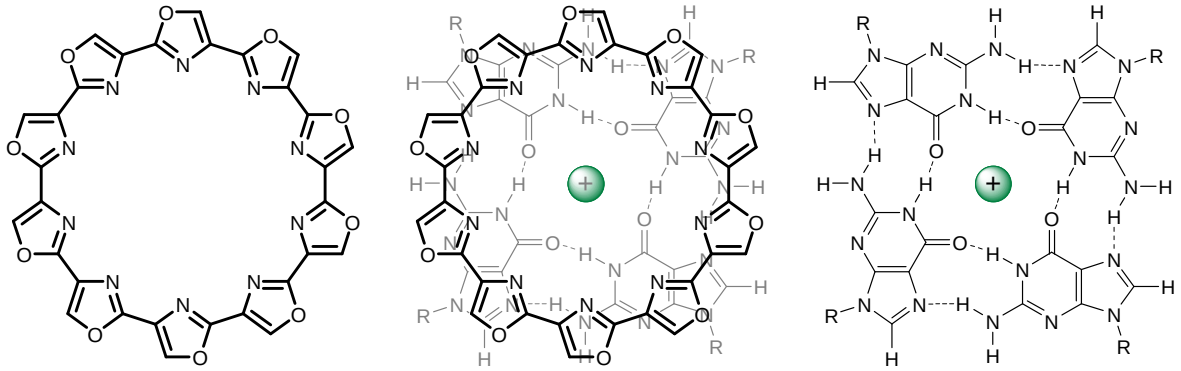


Figure 4.3: *Density Functional / SVWN/DN**-optimized geometries of telomestatin and its linear analog*



31

Figure 4.4: *[0.$_{10}$](2,4)1,3-Oxazolophan (**31**) on the G-tetrad*

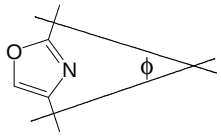


Figure 4.5: The calculated angle ϕ

Adding more oxazole units to the macrocyclic ring system is one possible option to reduce ring-strain. Another approach is to change the identities of the heterocycles to increase the angle ϕ. This can be achieved by exchanging the oxygen of the oxazole with other heteroatoms. Replacing O with NH, for example, does not make a large difference to the ring geometry since nitrogen is from the same period. Changing oxygen to sulphur however causes a significantly different orientation of the

substituents in 2- and 4-position, and a larger angle ϕ is predicted. This effect is even larger with selenium and the selenazole system. Simulations of imidazole, thiazole, and selenazole were performed (*Figure 4.6*) and compared to reported crystallographic data (*Table 4.1*).

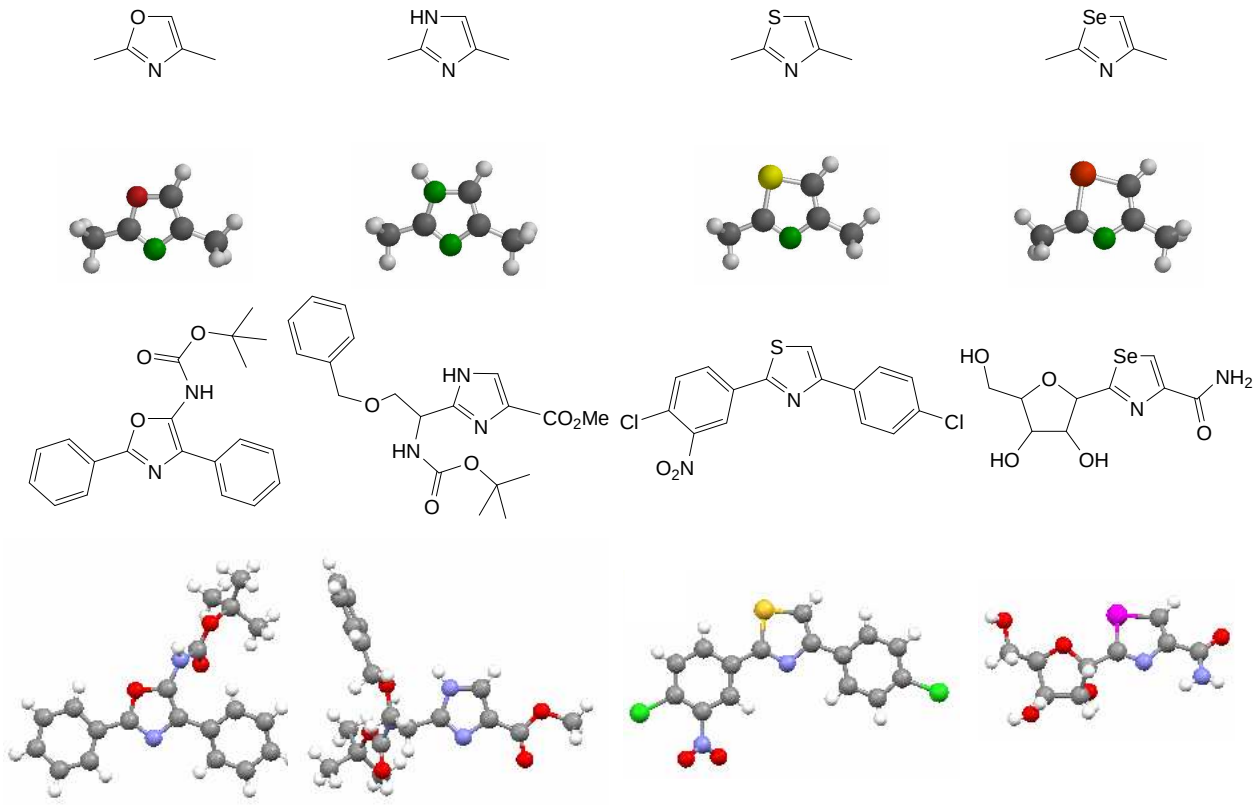


Figure 4.6: *Oxazole, imidazole, thiazole and selenazole as ChemDraw pictures (first row), as Density Functional / pBP/DN**-optimized geometries (second row), ChemDraw pictures of the used molecules in the crystal (third row), and the according crystal structure representations (fourth row)*

Table 4.1: *Calculations for angle ϕ*

	Oxazole	Imidazole	Thiazole	Selenazole
ϕ (simulated)	35°	39°	48°	51°
Expected ring units	10.2	9.1	7.5	7.0
ϕ (crystal)	36°	41°	49°	50°
Expected ring units	10.1	8.7	7.3	7.3

These calculations predict significant differencies between oxygen (and nitrogen) versus sulphur, but small differences between sulphur and selenium. While ten oxazoles are predicted to be required for closing a macrocycle, in theory, only seven or eight thiazole units are needed. A ring-strain simulation was conducted with

[0.$_8$](2,4)1,3-thiazolophan by cutting one bond connecting single thiazoles and re-optimizing the structure (*Figure 4.7*). The resulting structure does not open up as in the case of telomestatin (*Figure 4.3*).

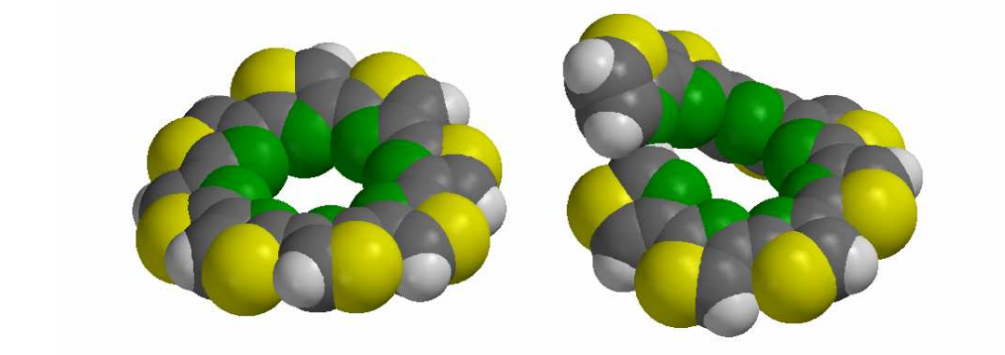


Figure 4.7: *Density Functional / SVWN/DN**-optimized geometry of [0.$_8$](2,4)1,3-thiazolophan*

4.3 Rational design of C_4-symmetric telomestatin analogs

As discussed in section 4.1, there are three structural features of telomestatin that can be improved: the symmetry, planarity, and predicted ring-strain. The topic of symmetry is easily addressed by designing a macrocycle composed of four identical ring segments giving C_4 symmetry. To reduce the ring-strain, half of the oxazoles will be replaced by thiazole units. Alternating oxazole and thiazole units should therefore provide planar C_4-symmetric molecules with little or no ring-strain (*Figure 4.8*).

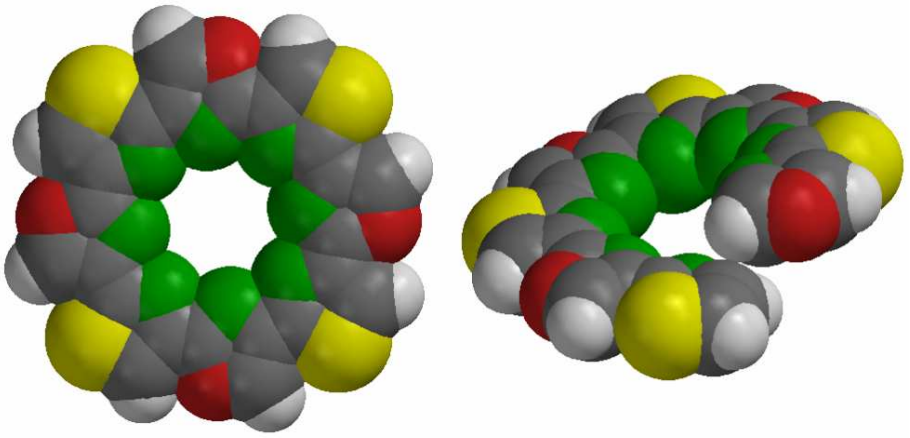


Figure 4.8: *Density Functional / SVWN/DN**-optimized geometry of [0.$_4$]{(2,4)1,3-thiazolo[0](2,4)1,3-oxazolo}phan and its linear analog*

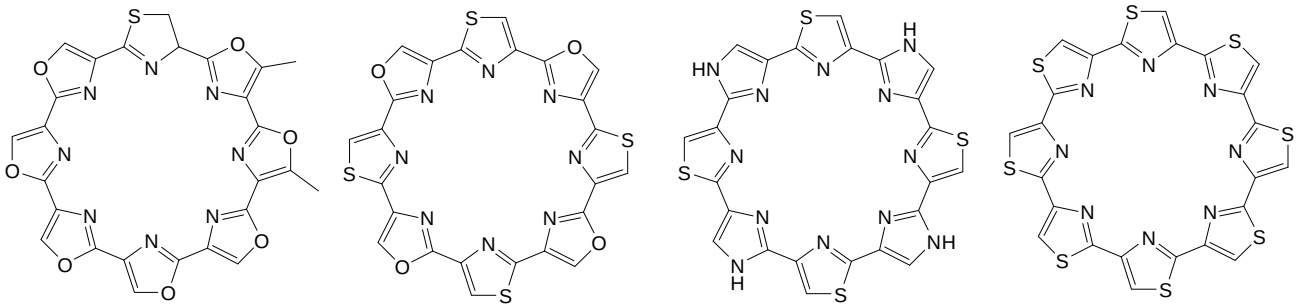
Calculations based on the angle ϕ (*Table 4.1*) suggest that a macrocycle containing four thiazoles together with four oxazole or imidazole units should have no ring-

strain. This should make the synthesis of such compounds more feasible. Imidazole units should have comparable angles as oxazole but will exhibit better solubility properties in water, making the [0.$_4$]{(2,4)1,3-thiazolo[0](2,4)-1*H*-1,3-imidazolo}phan an important target molecule. The NH groups of the imidazole units could also be used to attach different substituents, giving access to a series of water soluble telomestatin analogs.

Table 4.2: *Predicted total angles of macrocycles composed of variable heterocycles*

	4 $\phi_{(thiazole)}$ + 4 $\phi_{(oxazole)}$	4 $\phi_{(thiazole)}$ + 4 $\phi_{(imidazole)}$	8 $\phi_{(thiazole)}$	8 $\phi_{(oxazole)}$	10 $\phi_{(oxazole)}$
Calculations based on simulation	332°	348°	384°	280°	350°
Calculations based on crystal structure	340°	360°	392°	288°	360°

These calculations have motivated the synthesis of three target molecules: [0.$_4$]{(2,4)1,3-thiazolo[0](2,4)1,3-oxazolo}phan (4TOP, **32**) and [0.$_4$]{(2,4)1,3-thiazolo[0](2,4)-1*H*-1,3-imidazolo}phan (4TIP, **33**), which are likely to be planar systems, and [0.$_8$](2,4)1,3-thiazolophan (8TP, **34**) which will be slightly non-planar (*Figure 4.9*).



1 Telomestatin | **32** 4TOP | **33** 4TIP | **34** 8TP

Figure 4.9: *Three new telomestatin analogs as synthetic targets*

4.4 Specific questions and aims

With the design and synthesis of these three new telomestatin analogs, we aim to get three macrocyclic G-quadruplex ligands with improved the symmetry and shape as compared to telomestatin. Following synthesis, these three new macrocycles should allow us to answer the following three questions:

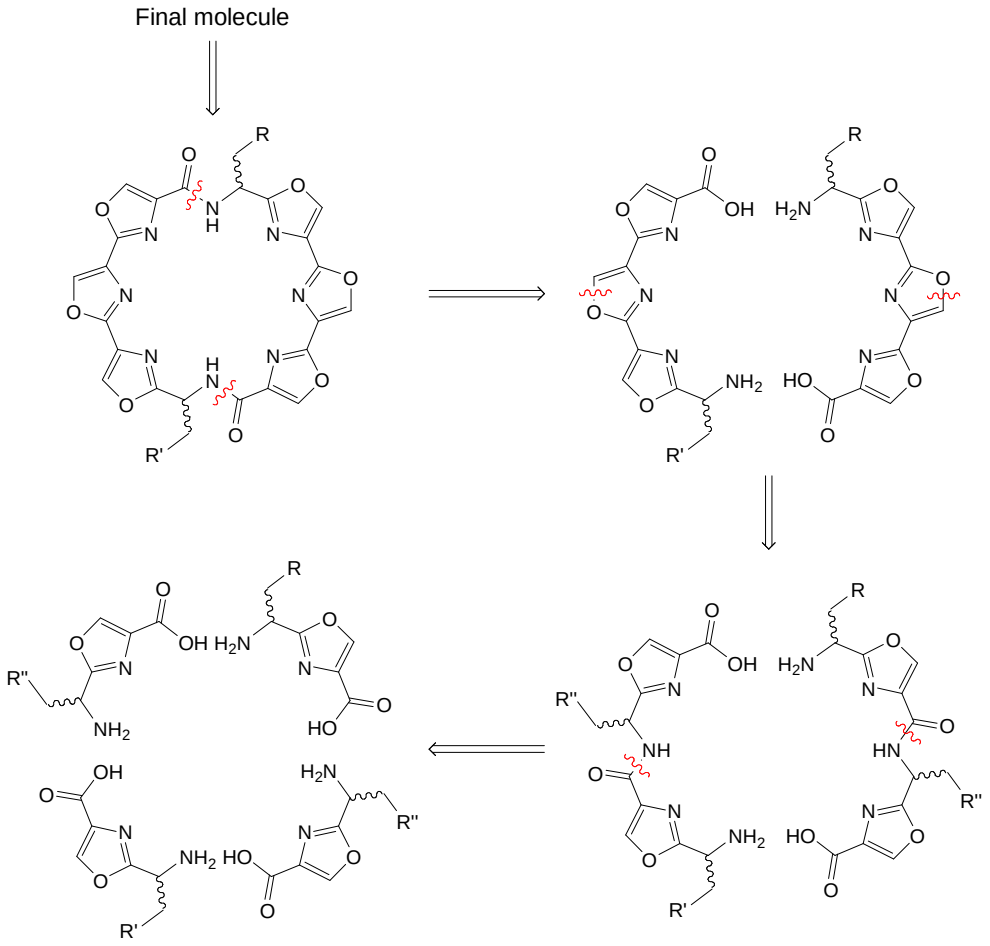
(1) Are cyclic polyazole systems with no or low ring-strain more synthetically accessible than those with high ring-strain?
(2) Are the proposed macrocyclic polyazoles planar?
(3) Do planar ring systems stack more tightly to G-tetrads and therefore provide improved binding properties for G-quadruplex structures?

Based on our computation analysis and experience with other G-quadruplex ligands, we predict the answer will be "yes" to all three questions. The following work was performed with the intension of providing an experiment-based assessment of these questions and predictions.

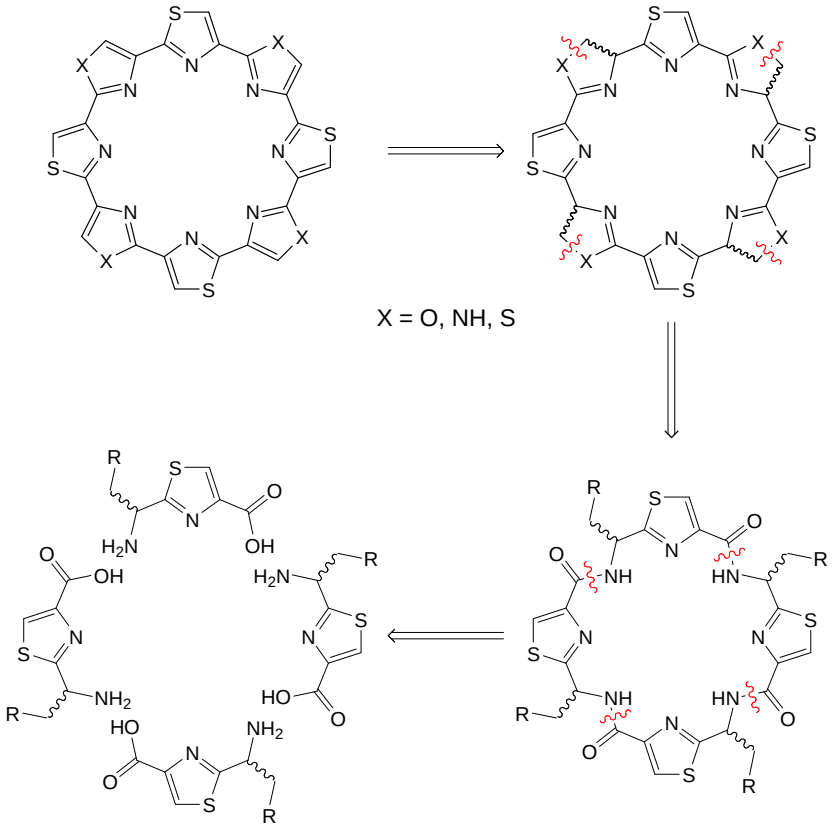
4.5 Retrosynthetic analysis

At the time this project was started the total synthesis of telomestatin was just published[157], as well as the first series of synthetic hexaoxazole-derivatives[162]. In both cases, the assembly of the two halfs of the macrocycle, each containing three oxazole units, was utilized as the basic strategy (*Scheme 4.1*). Since telomestatin has a C_1 and synthetic hexaoxazoles a C_2 symmetry, this is an efficient approach. In the case of C_4-symmetric target molecules, an alternative strategy was developed. As presented in *Scheme 4.2*, the target molecules containing eight azole units were reduced to four heterocycles connected by four amide bonds. The disconnected units can be seen as artificial amino acids containing a thiazole ring.

Scheme 4.1: *Published strategy for polyazole macrocycles*[157, 162]



Scheme 4.2: *Retrosynthetic analysis of C_4-symmetric macrocycles*



This proposed synthesis of a C_4-symmetric, macrocyclic scaffold from four identical subunits is highly economic. In theory, a single azole containing amino acid subunit can be used to synthesize a linear peptide followed by macrolactamization. The key step of this series is near the end of the sequence, where four azole rings are generated in one step. In the end, the development of novel synthetic methodology was needed to accomplish this challenging goal.

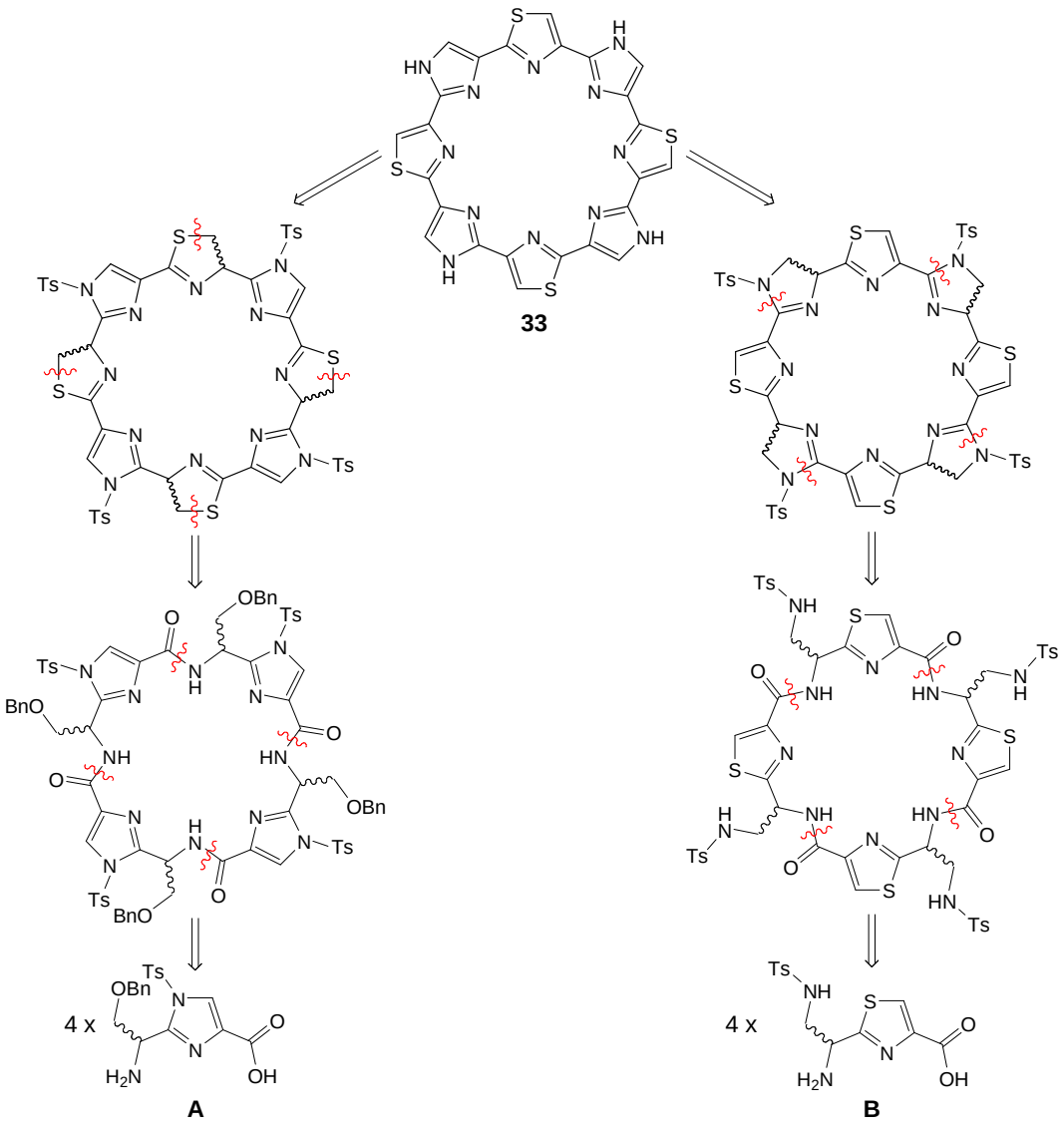
5. Synthesis of Telomestatin Analogs

5.1 Target 1: [0.$_4$]{(2,4)1,3-Thiazolo[0](2,4)-1*H*-1,3-imidazolo}phan (33)

5.1.1 Retrosynthetic analysis

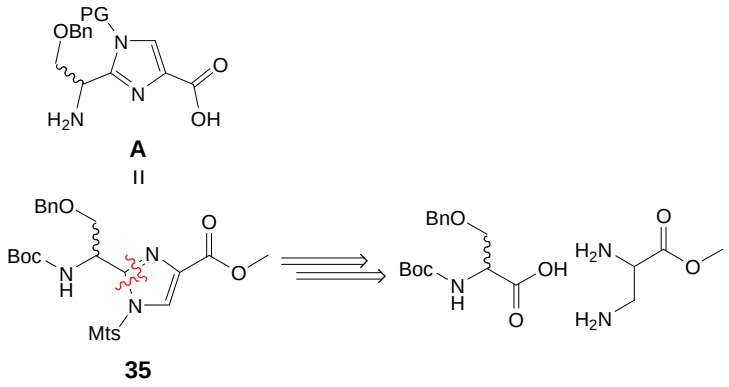
[0.$_4$]{(2,4)1,3-Thiazolo[0](2,4)-1*H*-1,3-imidazolo}phan (4TIP, **33**) is expected to be a planar macrocycle. Of the three proposed telomestatin analogs, macrocycle **33** is least likely to have any ring strain (*Table 4.2*). Upon protonation, the imidazole groups should increase the solubility of this compound in aqueous solutions. They also provide the opportunity for alkylation of the macrocycle, giving rise to a series of tetracationic, water soluble derivatives.

Scheme 5.1. *Two alternative retrosynthetic analyses of macrocycle **33***

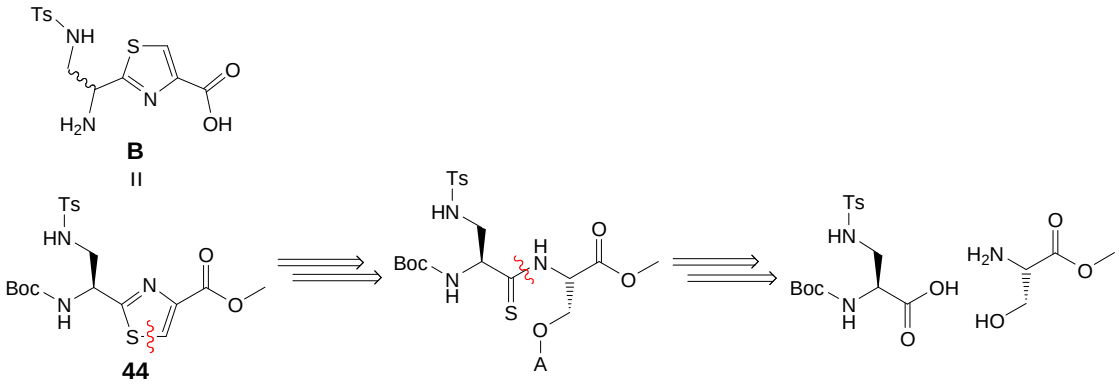


33 can be derived by assembling four units of an imidazole (**A**) or a thiazole (**B**) amino acid (*Scheme 5.1*). Imidazole amino acids of type "**A**" can be synthesised by fusing 2,3-diaminopropionic acid with serine (*Scheme 5.2*). Thiazole amino acids of type "**B**" can be prepared by coupling a 2,3-diaminopropionic acid with a serine residue, followed by thiazole formation on the dipeptide (*Scheme 5.3*).

Scheme 5.2. *Retrosynthesis of imidazole amino acids*



Scheme 5.3. *Restrosynthesis of thiazole amino acids (A = activating group)*



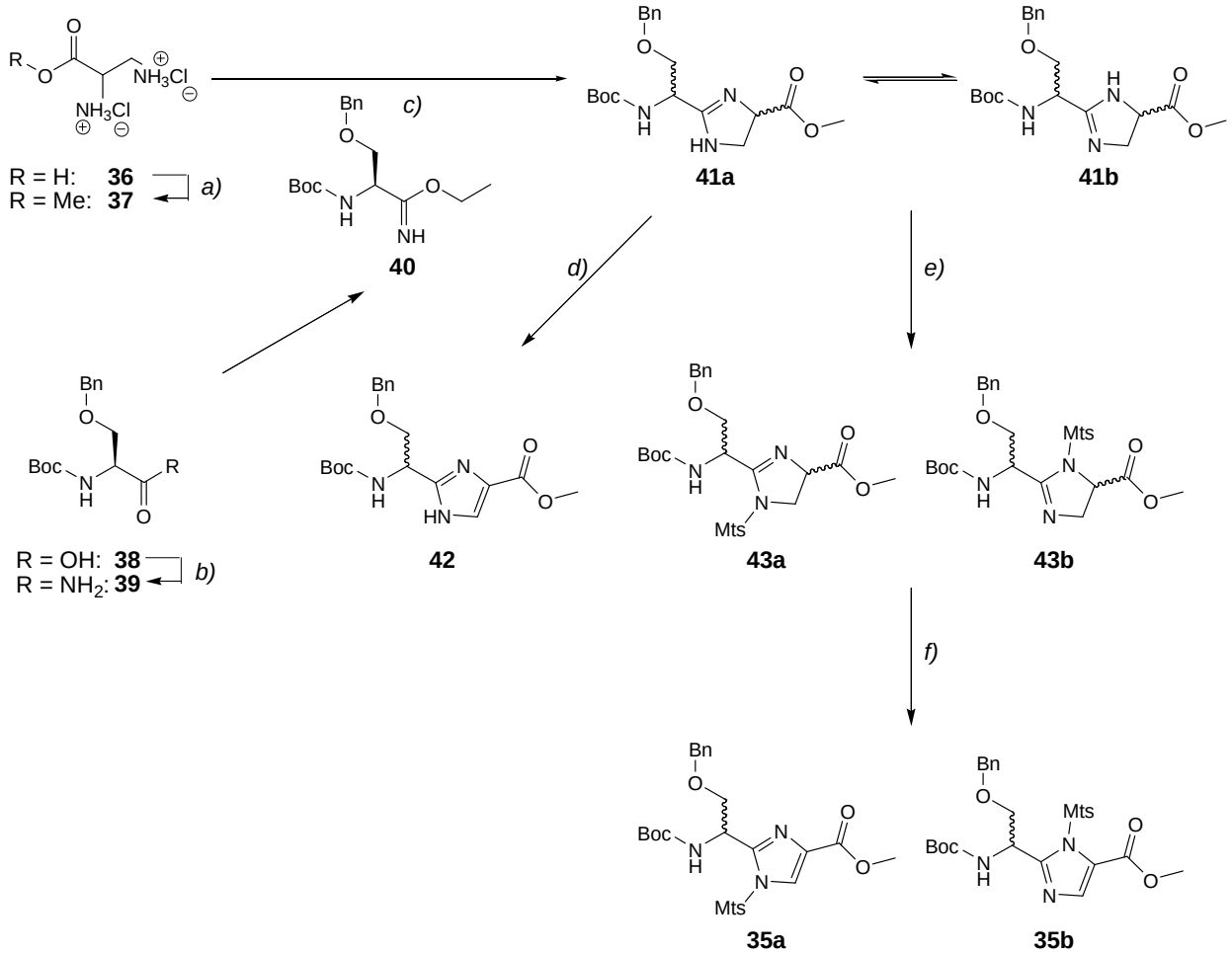
5.1.2 Synthesis of azole amino acids

5.1.2.1 Boc-I(NMts)Ser(OBn)-OMe (**35**)

The synthesis of methyl 2-{2-(benzyloxy)-1-[(*tert*-butoxycarbonyl)amino]ethyl}-1-mesityl-1*H*-imidazole-4-carboxylate (Boc-I(NMts)Ser(OBn)-OMe, **35a** / **35b**) was accomplished starting from two orthogonally protected amino acids (*Scheme 5.4*). 2,3-diaminopropionic acid dihydrochloride (**36**) was first esterified using $SOCl_2$ in MeOH[179] giving methyl 2,3-diaminopropionate dihydrochloride (**37**) in 97%. Separately, L-Boc-Ser(OBn)-OH (**38**) was converted to the corresponding amide using Boc_2O and $[NH_4][HCO_3]$ in dioxane to furnish L-Boc-Ser(OBn)-NH_2 (**39**) in a

yield of 92%[180, 181]. **39** was activated for nucleophilic attack by ethylation with "Meerwein salt" Et_3OBF_4. The resulting *O*-ethyl-(2*S*)-3-benzyloxy-2-[(*tert*-butoxycarbonyl)amino]propanimidoate (**40**) is very reactive towards water and is readily converted into the corresponding ethyl propionate. Compound **40** was therefore not isolated and reacted with the diamine **37** in a one-pot reaction in the presence of 2.5 equiv. Et_3N. Due to the basic reaction conditions, racemization of both stereogenic centers was observed. The mixture of diastereomers **41** was oxidized to the corresponding imidazole **42** using 10 equiv. of MnO_2 as an oxidazing agent[182] (*Scheme 5.4*). **42** could be produced in three steps with an overall yield of 27%. The structure of **42** was confirmed with x-ray crystallography (*Figure 5.1*).

Scheme 5.4. *Synthesis of the Imidazole containing amino acid **35***



*a) 7.5 equiv. $SOCl_2$, MeOH, 0℃, 20 min, 75℃, 5 h; b) 1.5 equiv. Boc_2O, 0.66 equiv. Pyridine, 1.25 equiv. $[NH_4][HCO_3]$, dioxane, rt, N_2, 16 h; c) 1. **39**, 1.1 equiv. Et_3OBF_4, 3 equiv. CH_2Cl_2, rt, Ar, 9 h; 2. 1.05 equiv. **37**, 2.5 equiv. Et_3N, 9, CH_2Cl_2, rt, N_2; d) 20 equiv. MnO_2, CH_2Cl_2, rt, 96 h; e) 2 equiv. MtsCl, 2 equiv. Et_3N, CH_2Cl_2, rt, N_2, 24 h; f) 1,1 equiv. DBU, 1.2 equiv. $BrCCl_3$, CH_2Cl_2, N_2, rt, 16 h.*

The two tautomeric forms of **41** can be captured through NH protection with the trimethyltoluenesulfonyl (mesityl) group for furnish **43a** and **43b**. Oxidation to the

corresponding imidazoles **35a** and **35b** was then performed using DBU and $BrCCl_3$ in CH_2Cl_2, giving in four steps an overall yield of 33%. In $^1H/^{13}C$ NMR spectra of **35**, only one type of signals can be found suggesting the formation of only one of the two possible forms, likely **35a**. This corresponds to position of NH in the single tautomer of *rac*-**42** observed in our crystal structure (*Figure 5.1*).

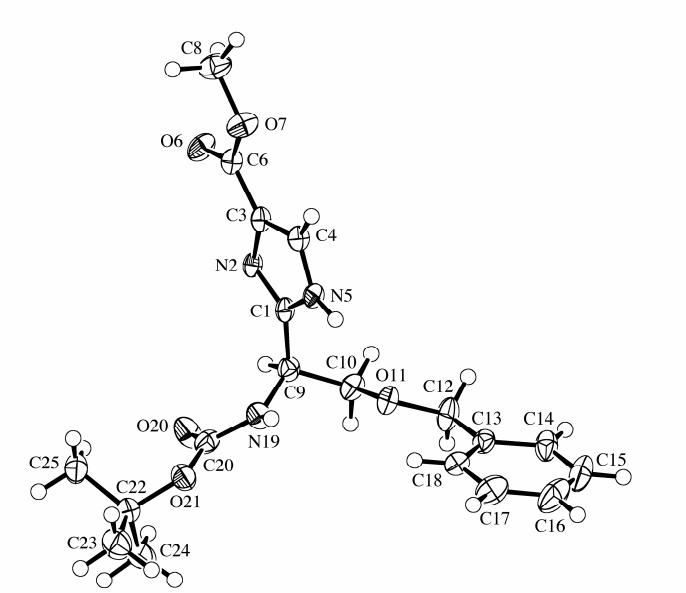


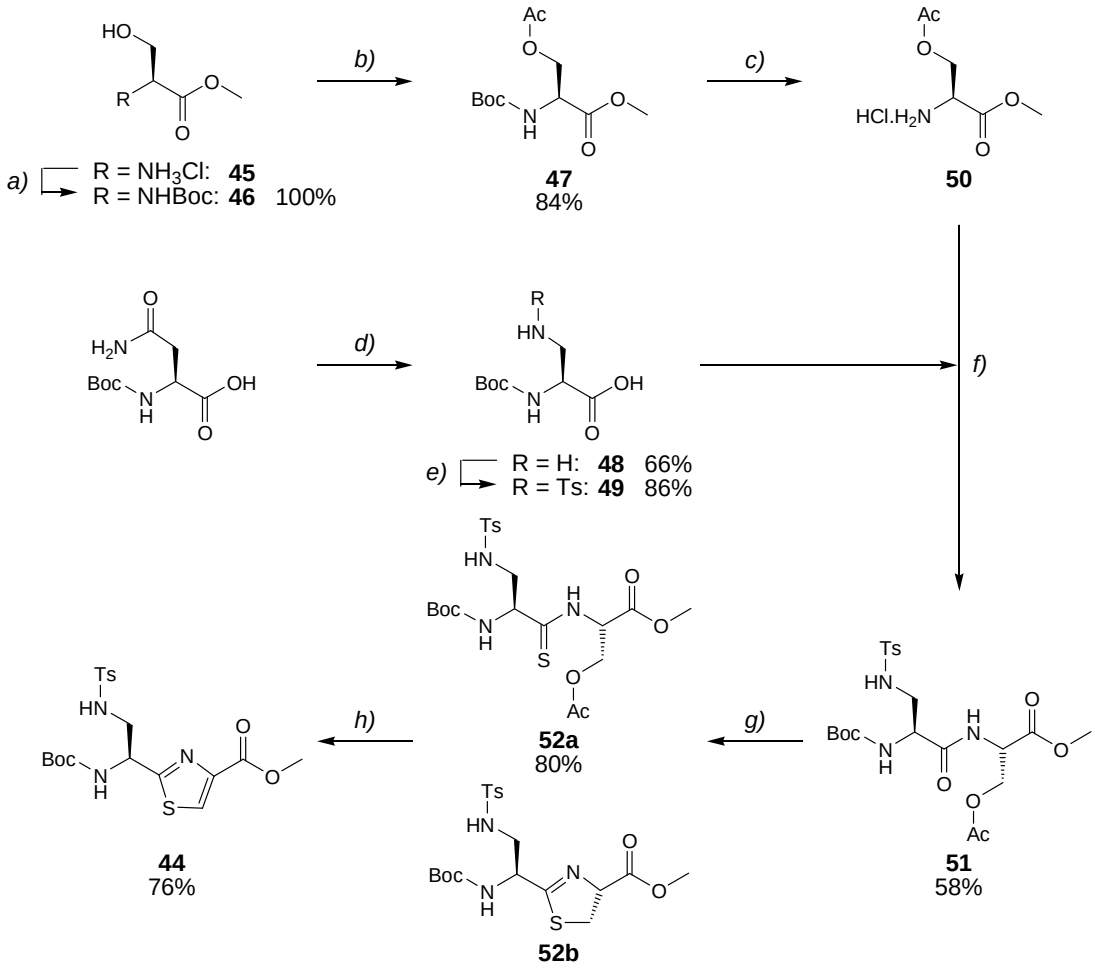
Figure 5.1: *ORTEP Plot of the molecular structure of* rac-***42*** (*50% probability ellipsoids)*

5.1.2.2 Synthesis of Boc-TDAP-OMe (**44**)

The thiazole containing amino acid methyl 2-{(1*S*)-1-[(*tert*-butoxycarbonyl)amino]-2-(tosylamino)ethyl}-1,3-thiazole-4-carboxylate (Boc-TDAP(NTs)-OMe, **44**) was synthesised starting with serine and asparagine (*Scheme 5.5*). Commercially available serine methylester (**45**) was protected with a Boc group, giving **46**, and with an acetate group on the alcohol to furnish **47**. Separately, *N*-boc-asparagine was reacted in a Hofmann type rearrangement using (diacetoxyiodo)benzene "PIDA"[183] to furnish 3-amino-2-[(*tert*-butoxycarbonyl)amino]propionic acid (Boc-DAP-OH, **48**) in 66% yield. The new amino group was tosylated[184] to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(4-toluenesulfonylamino)propionic acid (Boc-DAP(NTs)-OH, **49**) in 88% yield. Separately, **47** was deprotected using TFA to furnish amine **50**. Amino acid coupling was performed using TBTU, and the resulting amide carbonyl of **51** was selectively thionated using Lawesson's reagent in boiling toluene. Using precisely 0.5 equiv. of this reagent provides the thioamide **52a**[185] without affecting the carbonyl groups in the ester and carbamate functional groups. Typical reaction

times are two to three hours to give isolated yields of approximately 80%. Longer reaction times favor the formation of the thiazoline product **52b**. Using 3.5 equiv. DBU and 1.2 equiv. $BrCCl_3$, the mixture of thioamide **52a** and thiazoline **52b** is converted to thiazole **44**. Using this approach, **44** is synthesised with an overall yield of 23% in six steps (*Scheme 5.5*).

Scheme 5.5. *Synthesis of the thiazole-containing amino acid **44***



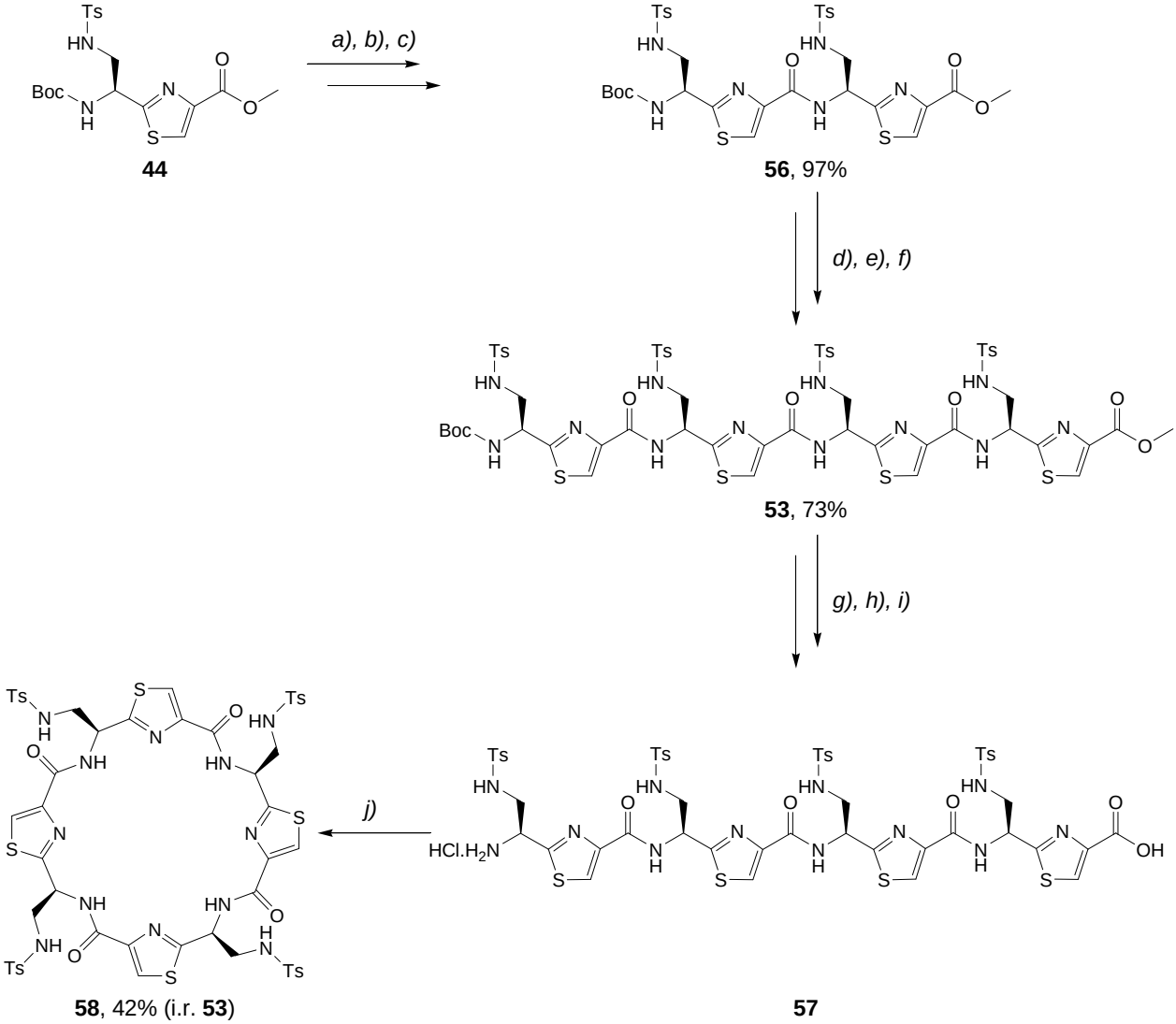
a) 1.1 equiv. Boc_2O, 2.2 equiv. Et_3N, dry CH_2Cl_2, 0°C, N_2, 3 h; b) 1.5 equiv. Ac_2O, 2 equiv. Et_3N, 3 mol% DMAP, CH_2Cl_2, 0°C to rt, N_2, 1.5 h; c) 1. TFA/CH_2Cl_2 1:3, rt, N_2, 3 h; 2. Dowex anion exchange resin, MeOH, rt, N_2, 16h; d) 1.2 equiv. PIDA, AcOEt/MeCN/H_2O 2:2:1, 15°C, 30 min, rt, 16 h; e) 1.05 equiv. TsCl, MeCN/H_2O/aq. sat. $NaHCO_3$ 4:1:1, 0°C to rt, 6 h; f) 1 equiv. TBTU, 5 equiv. NMM, CH_2Cl_2, rt, N_2, 16 h; g) 0.5 equiv. Lawesson reagent, toluene, reflux, N_2, 2 h; h) 3.5 equiv. DBU, 1.2 equiv. $BrCCl_3$, rt, N_2, 3 h.

Building block **35** is potentially a regioisomeric mixture and a racemate (*Scheme 5.4*). In contrast, building block **44** is an enantiomerically pure substance (*Scheme 5.5*). **44** was selected as the primary building block for [0.$_4$]{(2,4)1,3-thiazolo[0](2,4)-1*H*-1,3-imidazolo}phan (4TIP, **33**) so that all intermediates are generated in enantiomerically pure forms.

5.1.3 Synthesis of Boc-[TDAP(NTs)]$_4$-OMe (**53**)

The oligomerization of building block **44** was accomplished using standard amino acid chemistry (*Scheme 5.6*). **44** was selectively deprotected, either in MeOH/LiOH or with TFA in CH_2Cl_2. The resulting free acid (**54**) and amine (**55**) were coupled to furnish dimer **56** using either DCC, PyBroP or TBTU as coupling reagent. For optimal yield and purification reasons (97% isolated yields), TBTU was selected to scale up the reaction.

Scheme 5.6. *Synthesis of the macrocycles **58***



*a) **44**, 5 equiv. LiOH, MeOH/H_2O 1:1, rt; b) **44**, TFA/CH_2Cl_2 1:2, rt, 3 h; c) 1 equiv. TBTU, 5 equiv. Et_3N, CH_2Cl_2, rt, 16 h; d) **56**, 6 equiv. LiOH, MeOH/H_2O 3:1, rt; e) **56**, TFA/CH_2Cl_2 3:1, rt, 6 h; f) 1.5 equiv. PyBOP, 6 equiv. NMM, DMF, rt, N_2, 16 h; g) 6 equiv. LiOH, hot MeOH/H_2O 2:1 (c = 4 mM), rt; h) TFA/CH_2Cl_2 1:1, rt, N_2, 6 h; i) Anion exchange: Dowex, MeOH, rt, 16 h; j) 5 equiv. PyBOP, 20 equiv. NMM, DMF (c = 1.5 mM), rt, N_2, 16 h.*

Dimerization of **56** delivered tetramer **53** in analogous way (*Scheme 5.6*). The key coupling step was achieved in better yield when 1.5 equiv. PyBOP were used in DMF. With tetramer **53**, solubility started to become limited in solvents like AcOEt, $CHCl_3$ and MeOH. Very strong solvents like DMF or DMSO were needed to fully dissolve this peptide-like product.

5.1.4 Macrolactamization

Macrolactamization was first conducted in CH_2Cl_2 with 1 equiv. TBTU and yielded around 30% of the macrocycle. Reactions were also attempted in DME with 3 equiv. HOBt and 1.2 equiv. of diisopropylcarbodiimide or with 4 equiv. DPPA as the coupling reagent[155]. These two procedures were not efficient for lactamization: only traces of the desired product could be detected on TLC. Optimised yields in the range of 40 and 50% were obtained using a 1.5 mM solution of **57** in DMF, 10 equiv. of NMM and 5 equiv. PyBOP (*Scheme 5.6*). The isolated product was consistent with the stereochemically pure "all-*S*"-**58**. ^{1}H-NMR indicates exactly one set of overlapping signals for all of the four repeating units (*Figure 5.2*). As a side product, another slightly less polar substance could be isolated. According to ESI-MS data it contains macrocycle **58**, but the ^{1}H-NMR indicates a complex mixture, showing at least four sets of signals (*Figure 5.3*). This suggests that a small amount of racemization occurs during the macrolactamization reaction.

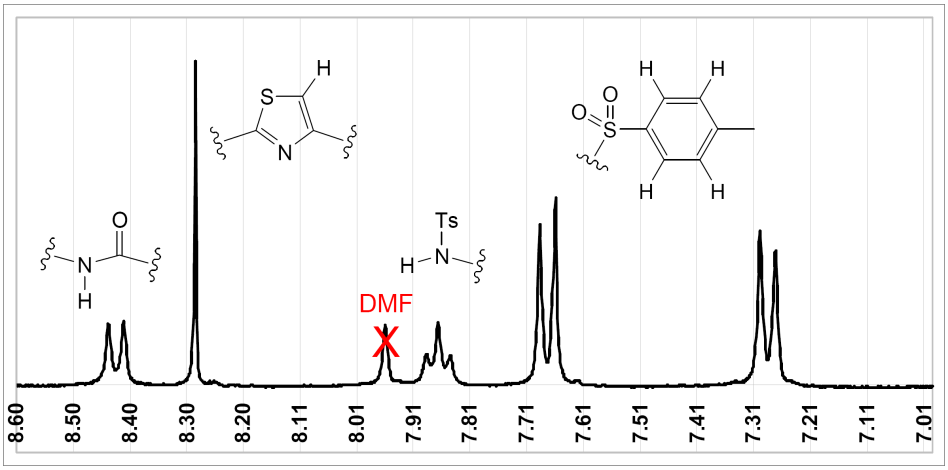


Figure 5.2: *^{1}H-NMR of the all-S-**58** in DMSO*

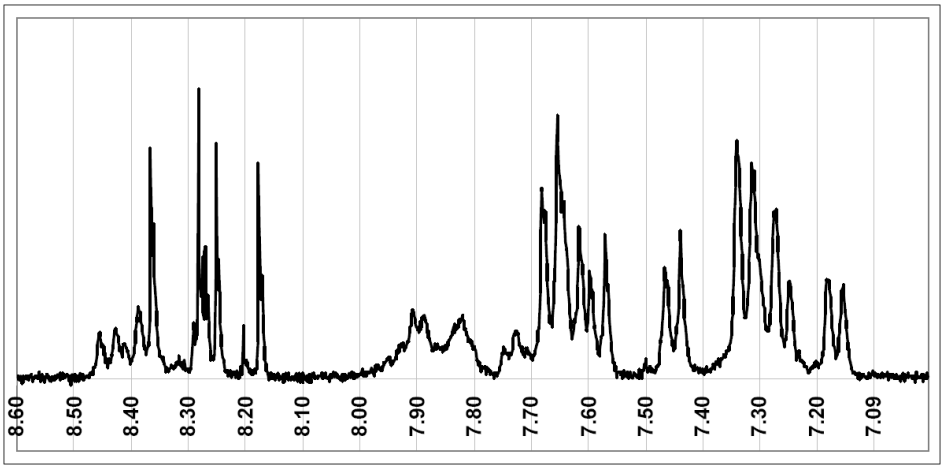
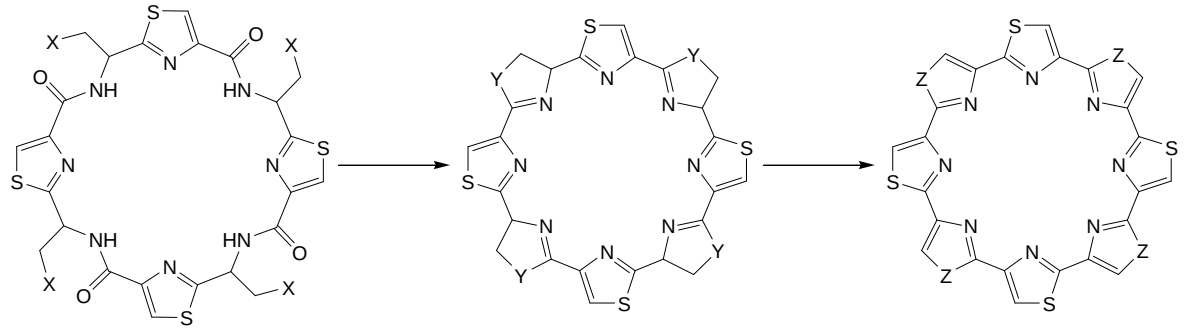


Figure 5.3: *^{1}H-NMR of a mixture of diastereoisomers of **58** in DMSO*

5.1.5 Strategies for formation of imidazole units

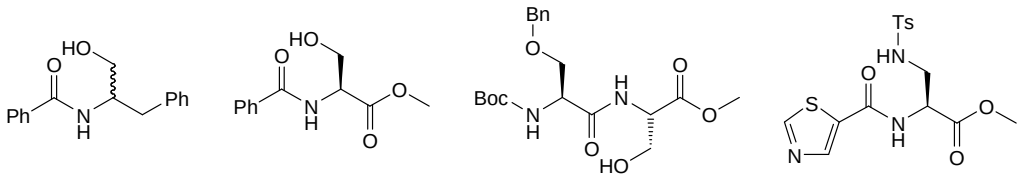
Macrocycle **58** requires three additional reactions to be converted into target compound 4TIP (**33**) (*Scheme 5.7*): cyclodehydration, oxidation, and deprotection the tosyl groups. Due to the limited solubility and/or folding properties of these compounds, these steps were highly challenging. According to ^{1}H-NMR, compounds **60** and **58** adopt 3-dimensional structures in organic solvents like MeOH, as indicated by complex and broadened signals.

Scheme 5.7. *Final modifications on the macrocycles*



To test possible reaction conditions for imidazole formation, several test molecules were synthesized (*Figure 5.4*). Compounds **63** and **64** are very robust models for reactions starting with β-hydroxyamides. As compared to the macrocycle, a π acceptor attached to the NHCH is missing in **63**, this CH is therefore much less acidic than in the macrocycle. This lack of an acidic CH was corrected in molecule **64**. The ester group in this compound is a better π-acceptor than a thiazole unit and is also

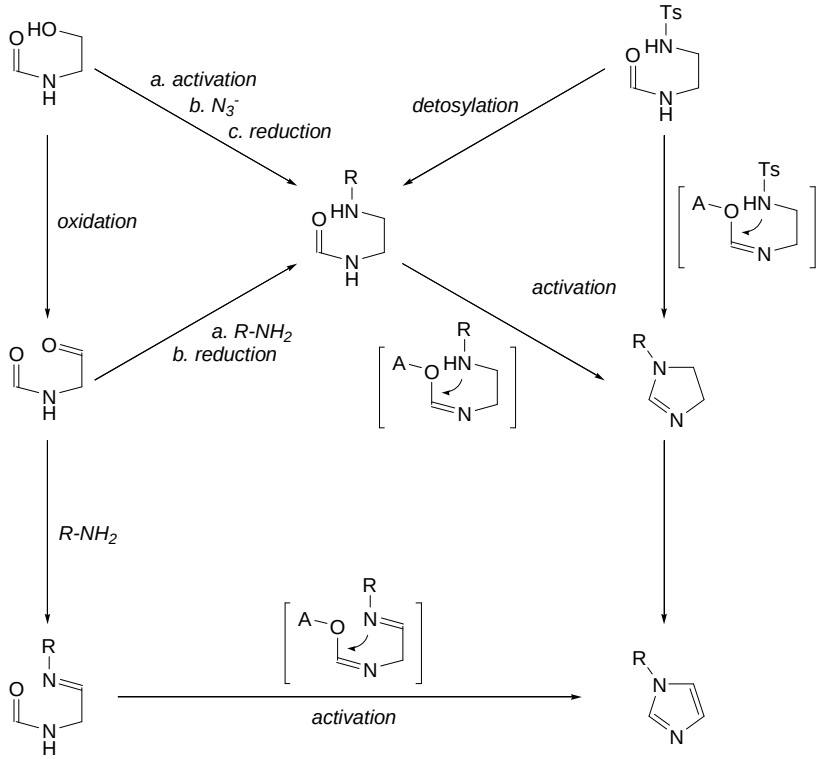
more reactive toward nucleophilic attack. Diserine **65**, with its additional functional groups, is a more sensitive and reactive system. **66** is the most similar test substrate to macrocycle **58**. Last, but not least, the linear building blocks **56** (and **53**) can also be used as model compounds.



63 **64** **65** **66**

Figure 5.4: *Different model compounds used for test reactions*

Scheme 5.8: *Strategies for the formation of imidazolines and imidazoles*



β-Hydroxy- and β-(tosylamino)amides require different strategies for ring-closure (*Scheme 5.8*). Activation of the amide oxygen and attack by the tosylamine delivers the imidazoline, which is then oxidized to the imidazole. A more reactive primary amine for ring-closure could be provided by deprotection of the amine, or alternatively, by converting a primary hydroxyl into a primary amine. All of these paths require an oxidation as the last step. Alternatively, an amide and aldehyde can react to give imidazole in one step.

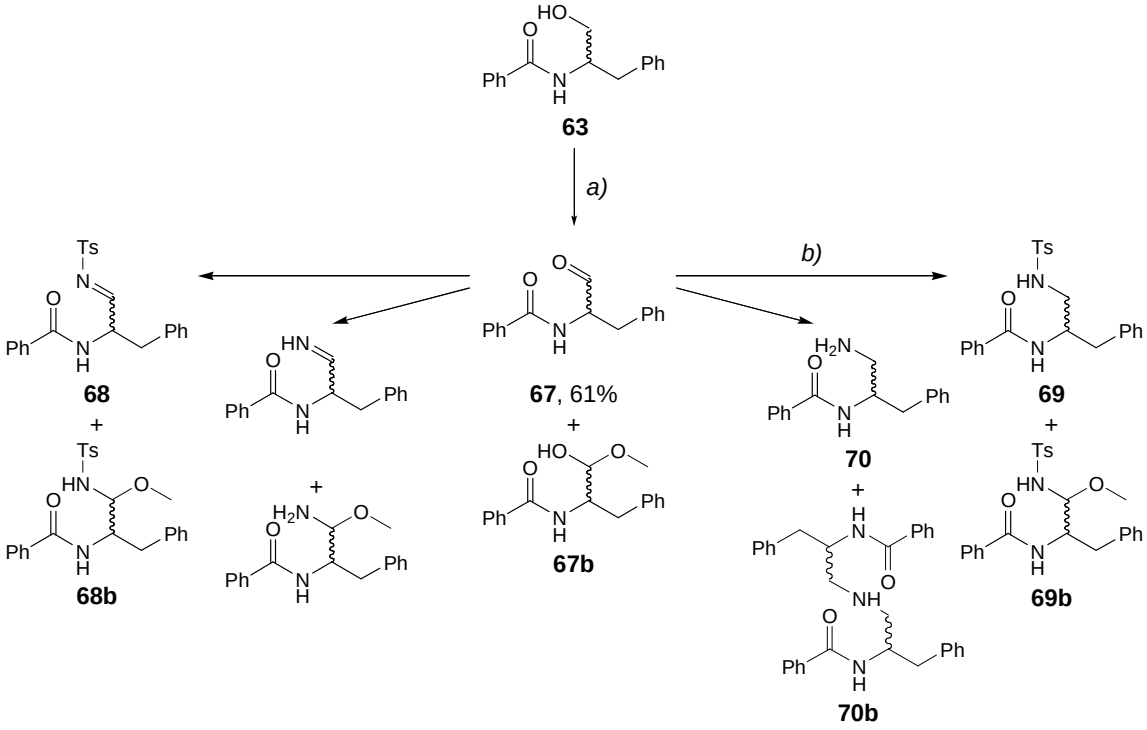
In the case of macrocycle **58**, with its four β-(tosylamino)amides, cyclodehydration reactions were envisioned to provide the tetraimidazoline macrocycle **62**. One challenge to this approach is the selective activation of the amide oxygen for elimination, while not affecting the sulfonyl oxygens.

5.1.6 Results and discussion

5.1.6.1 Using β-hydroxyamides to synthesize imidazolines

a. Aldehydes as intermediates to imidazoles

Scheme 5.9: *Aldehyde, imine formation and reductive amination*



a) 1.15 equiv. Dess-Martin, CH_2Cl_2, 0℃ to rt, N_2, 16 h;
b) 2 equiv. $TsNH_2$, 2.2 equiv. $NaBCNH_3$, MeOH, 0℃ to rt, N_2.

By using Dess-Martin periodinane in CH_2Cl_2, model compound **63** was converted into the corresponding aldehyde **67** in 61% isolated yield. This aldehyde is quite reactive and adds to MeOH almost quantitatively to give **67b**, according to ESI-MS. Aldehyde **67** was reacted in a 1:1 mixture of CH_2Cl_2 and MeOH and 0.2 equiv. Et_3N with $TsNH_2$[186], resulting in a mixture of **67** and **68** and each corresponding MeOH adduct **67b** and **68b**. Reductive amination of **67**, with tosylamide or ammonia in the presence of $NaBCNH_3$ furnished mixtures containing products **69** and **70**. The main

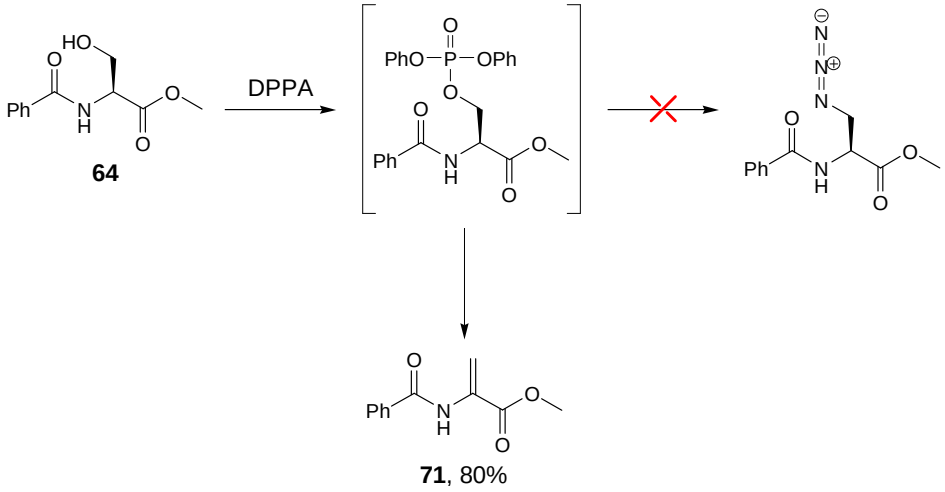
byproduct in the case of $TsNH_2$ was again the MeOH adduct **69b**. In the case of ammonia dimer **70b** was the main product (*Scheme 5.9*).

Oxidation followed by reductive amination was then tried on more difficult test substrate, **65**, using the same procedure. According to TLC, oxidation could be observed, but the product though was not stable enough to be isolated and characterised. Reductive amination was therefore attempted on the crude mixture, but many side products were observed by TLC. The desired product could be identified, but the yield was too low to pursue this strategy in the context of the macrocycle.

b. Reacting alcohol with azide to synthesize imidazolines

β-Azidoamides can be used in Aza-Wittig reactions to prepare imidazolines[187]. Substitution of the alcohol of **64** using DPPA was therefore attempted. Using reported reaction conditions[188], the elimination product **71** was obtained in yields ranging between 80 and 90% (*Scheme 5.10*). The desired azido-product was not observed.

Scheme 5.10. *Formation of the dehydroproduct **71***



c. Using oxazolines as intermediates towards imidazolines

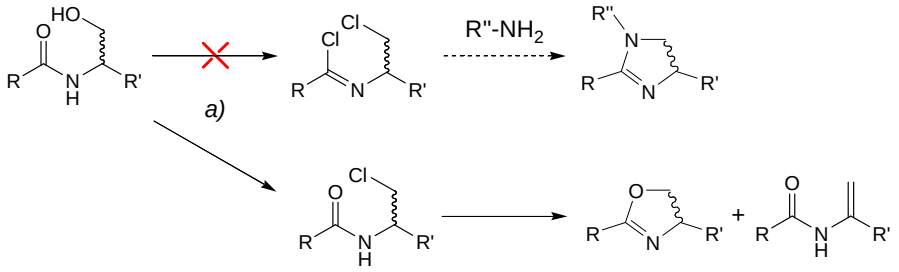
Oxazolines can be opened using sulphur or thiols as described in chapter 5.2.7.2. Since oxazolines were easily accessible on the level of test substrates (see chapter 5.3), ring opening was tried using amines to give β-hydroxyamidines. Accordingly, oxazoline **72** was dissolved in CH_2Cl_2, 1.25 equiv. $TsNH_2$ and 2.5 equiv. Et_3N were

added. The mixture was stirred for two days at rt under N_2 but no reaction could be observed. This strategy was therefore not further persued.

d. Activation of amide oxygens by chlorination

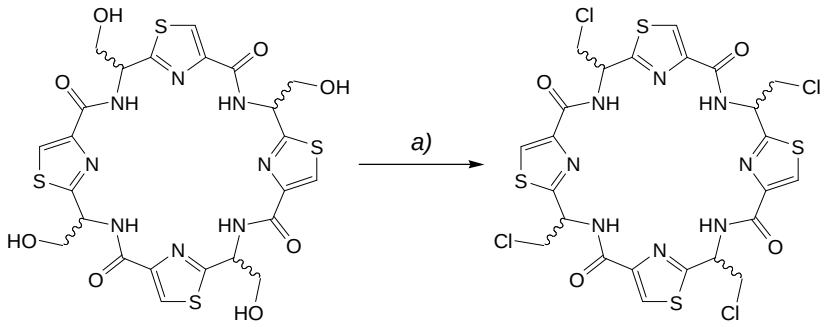
Double chlorination of the β-hydroxyamide was attempted according to published procedures by Boland[189] or Menges[190]. According to these procedures, it should be possible to convert an isolated dichloride intermediate into imidazoline by refluxing it with a primary amine. Unfortunately, in the case of our test molecule **64** we could only isolate the β-chloroamide **73** (see also chapter 5.3.3.1). This same transformation was also possible using DAST in a 0.5 M LiCl solution in THF. This latter method is much more gentle, and also applicable to the macrocycle. It was possible to obtain the tetrachloride **74** using this procedure in 30% yield (*Scheme 5.12*).

Scheme 5.11: *Chlorination, double chlorination and ring formation*



a) $SOCl_2$, reflux, N_2, 16 h or 1.5 equiv. DAST, 0.5 M LiCl in DMF, 0℃ to rt, N_2

Scheme 5.12: *Chlorination of **59***



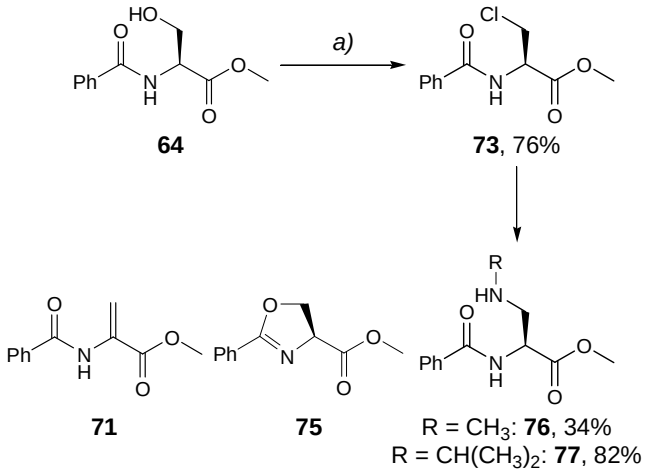
59 **74**, 30%

a) 4 x 1.5 equiv. DAST, 0.5 M LiCl in DMF, 0℃ to r t, N_2, 4 h

Attempts were made to react chloride-containing test substrates with benzylamine, isopropylamine and methylamine. Performing these reactions under basic conditions in the presence of Et_3N[189, 190], ring-closure to the oxazoline **75** and elimination to **71**

were observed, and none of the amine-substituted products could be identified. In the absence of Et_3N, the desired β-aminoamides could be synthesized by adding a primary amine. By performing the reaction at 0°C there was only a trace of the undesired elimination product observed. The reaction could be accomplished using methylamine or isopropylamine giving **76** in 34% and **77** in 82% (*Scheme 5.13*). The reaction was then attempted with the macrocyclic tetrachloride **74**. Following aqueous workup, a complex mixture of products was obtained, including the tetraalcohol **59** and multiple oxazoline units towards **61** or **78**, and also one or two additions of $MeNH_2$. Given this complex product distribution, this strategy was not pursued further.

Scheme 5.13: *Chlorination and substitution of the β-alcohol*



a) 1.5 equiv. DAST, 0.5 M LiCl in DMF, 0°C to rt, N_2

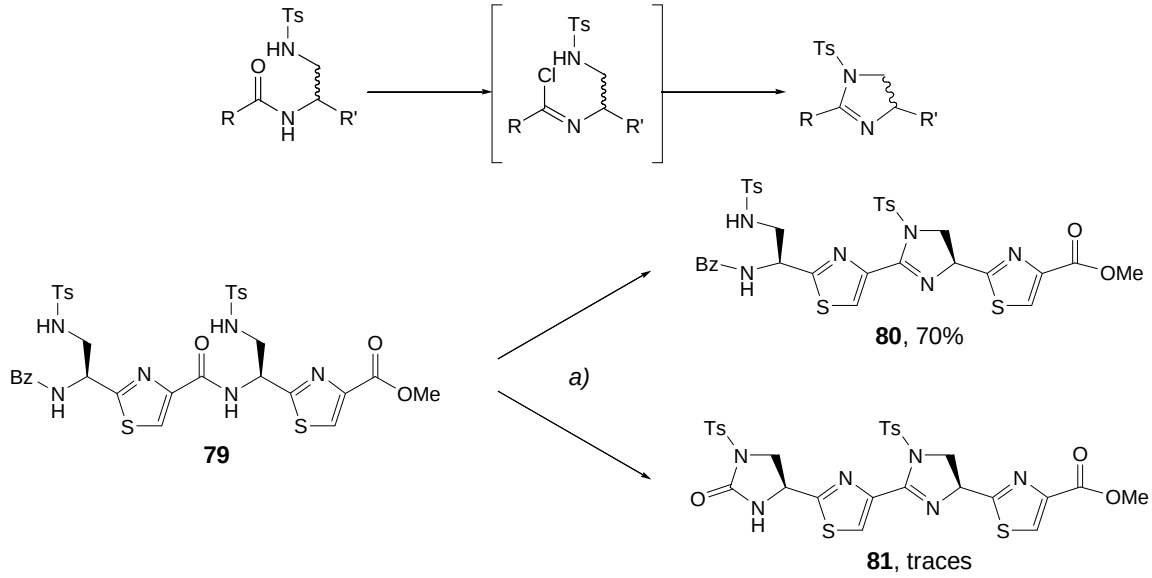
5.1.6.2 Using β-(tosylamino)amides to synthesize imidazolines

a. Activation of the amide oxygens by chlorination

Starting with β-(tosylamino)amide compounds, the chlorination of the amide should be an alternative route to enable the tosylated amino group to attack and close the imidazoline ring (*Scheme 5.14*). The reaction was tested on the dimer **56** and its respective benzoyl-derivative **79**. Performing the reaction in MeCN with 5 equiv. $POCl_3$ and heating at 100°C for three hours gave a mixture of starting material, the desired product **80** in about 70% yield and traces of byproduct **81**. Since the ring-closure worked reasonable well, the chlorination reaction was attempted using macrocycle **58**. It was dissolved in a 1:1 mixture of MeCN and $POCl_3$ and stirred at 75°C overnight and heated to 100°C for two hours. After careful aqueous workup,

ESI-MS revealed a mixture of all steps of water elimination, including a trace of the product **62** with 1221.3 ($[M_{\mathbf{62}} + H]^+$) (*Figure 5.5*). Attempts were made to optimize this reaction. Variable equivalents of $POCl_3$, temperature, and reaction times were screened. Alternative chlorination reagents like PCl_3, PCl_5, $SOCl_2$, $(COCl)_2$ were also evaluated. Attempts were made to increase reactivity by adding pyridine and Et_3N, but lowered yields and loss of the tosyl group were observed. In some cases, there was even elimination or direct ring-closure with formation of oxazoline byproducts. Apparently, under basic conditions, the differences between the reactivities of the amide and sulphonamide are not large enough to give good selectivity with these chlorinating reagents.

Scheme 5.14: *Chlorination of the amide to realise imidazoline formation*



a) 5 equiv. $POCl_3$, MeCN, 100°C, N_2, 3 h.

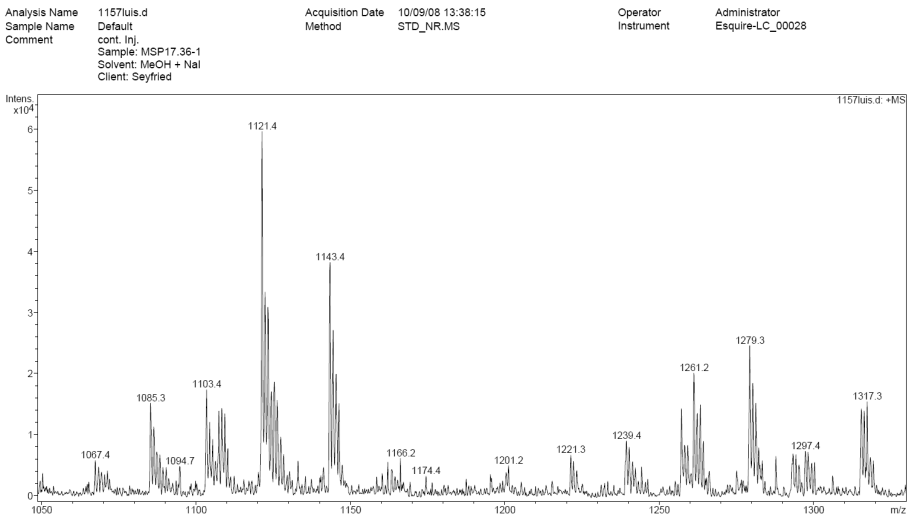


Figure 5.5. *ESI-MS of partial reactions to **62***

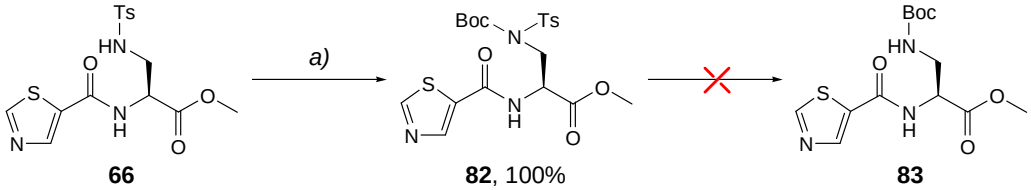
b. Using Mitsunobu conditions to activate amide oxygens

Starting with the β-(tosylamino)amide compounds, Mitsunobu-activation of the amide-oxygen should be an alternative route to close the imidazoline ring. Macrocycle **58** was dissolved in a saturated solution of LiCl in THF and DEAD and Ph_3P were added[191]. Only partial dehydration towards **62** was observed.

c. Detosylation of amines

The *p*-toluenesulfonyl protecting group attached to a primary amine is quite difficult to remove. In literature, only few examples are described. One is the removal of the tosyl group by ethanthiol[192]. According to this procedure, **66** was reacted with 6 equiv. K_2CO_3 and 1 equiv. ethanthiol in MeCN at rt. After two weeks no change could be observed on TLC. A second deprotection procedure first adds a Boc group to the tosyl protected amine to facilitate detosylation[193]. The diprotected test substrate **82** was then dissolved in MeOH and sonicated together with powdered Mg[194, 195]. The starting material is consumed but a mixture of different substances was obtained and the desired product **83** could not be detected (*Scheme 5.15*). It is expected that the thiazole ring is also susceptible to reduction and that a one electron reduction may initiate decomposition of the compound.

Scheme 5.15: *Expected detosylation from the diprotected β-aminoamide*



66 **82**, 100% **83**

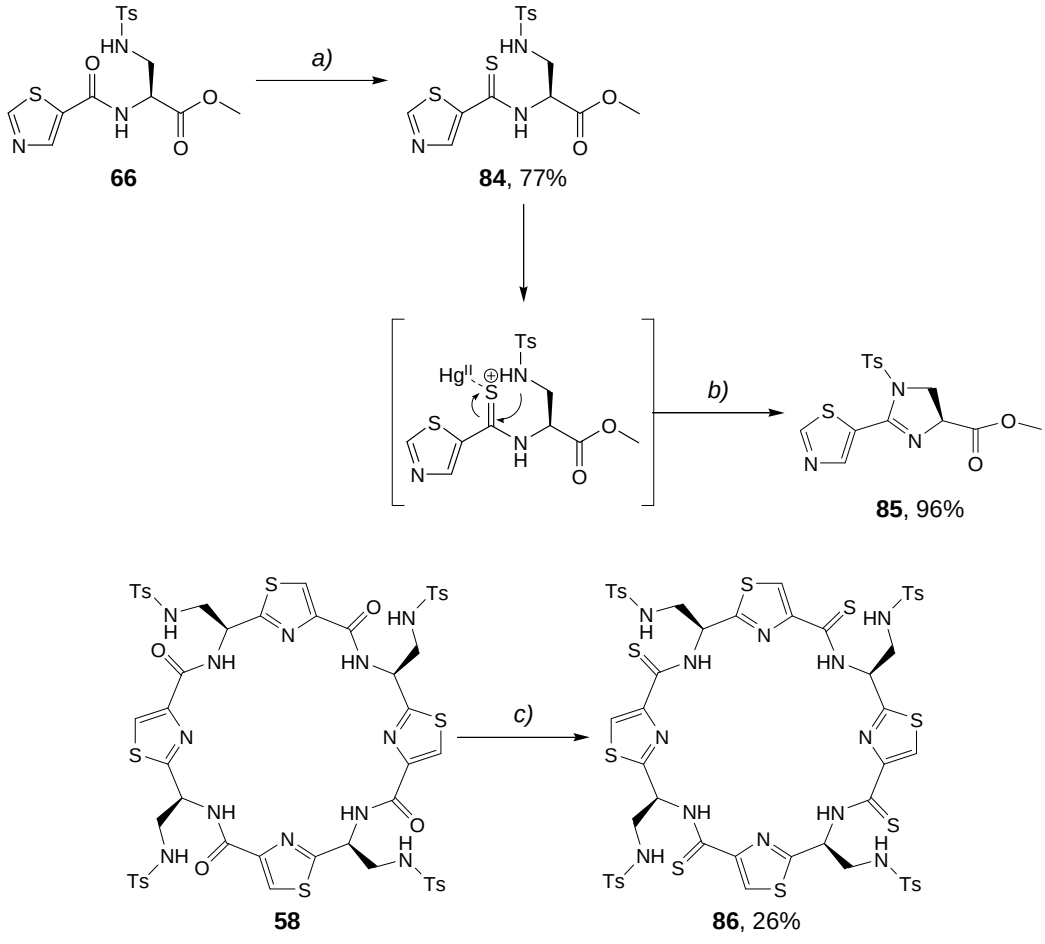
a) 1.1 equiv. Boc_2O, 0.1 equiv. DMAP, MeCN, rt, N_2, 4 h

d. Activation of the amide oxygens by thionation

Gavin and Hill[196] describe another, yet infrequently used method for imidazoline synthesis. They convert the amide to the thioamide and then activate the sulphur by complexation with mercury (*Scheme 5.16*). This procedure was first evaluated using model compound **66**. Thionation was performed using two equivalents of P_2S_5 in refluxing pyridine for 4.5 h. The resulting thioamide, **84**, was isolated in about 75% yield. Ring-closure was attempted in refluxing EtOH with one equiv. of HgO and Et_3N according to the published procedure by Ewin[196]. Under these conditions, a mixture of products was observed on TLC. This reaction was also attempted in pyridine or

with $Hg(TFA)_2$ as source of Hg^{2+}, but this did not improve the yield. A very good yield of 96% for **85** was obtained using 1.2 equiv. HgO and Et_3N in hot pyridine (100℃) for 2h. The thionation reaction was then conducted using macrocycle **58** using 20 equiv. of P_2S_5 and refluxing the solution for 5 h at 150℃. Under these conditions, a 26% yield of the tetrathioamide **86** was achieved. The mercury-promoted cyclization reaction was attempted in EtOH and pyridine using Et_3N as base, but failed to give the desired product. It is possible that due to secondary structures reactions across the ring did occur that lead to decomposition.

Scheme 5.16: *Thionation followed by activation with mercury*

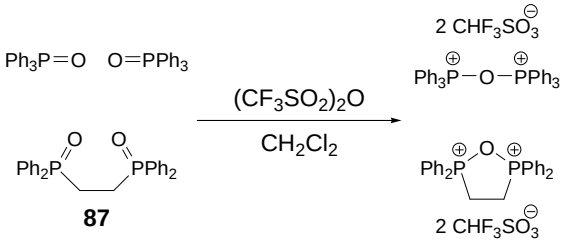


a) 2 equiv. P_2S_5, pyridine, reflux, N_2, 2 h; b) 1.2 equiv. HgO, 1.2 equiv. Et_3N, pyridine, 100℃, N_2, 2 h; c) 20 equiv. P_2S_5, pyridine, reflux, N_2, 5 h.

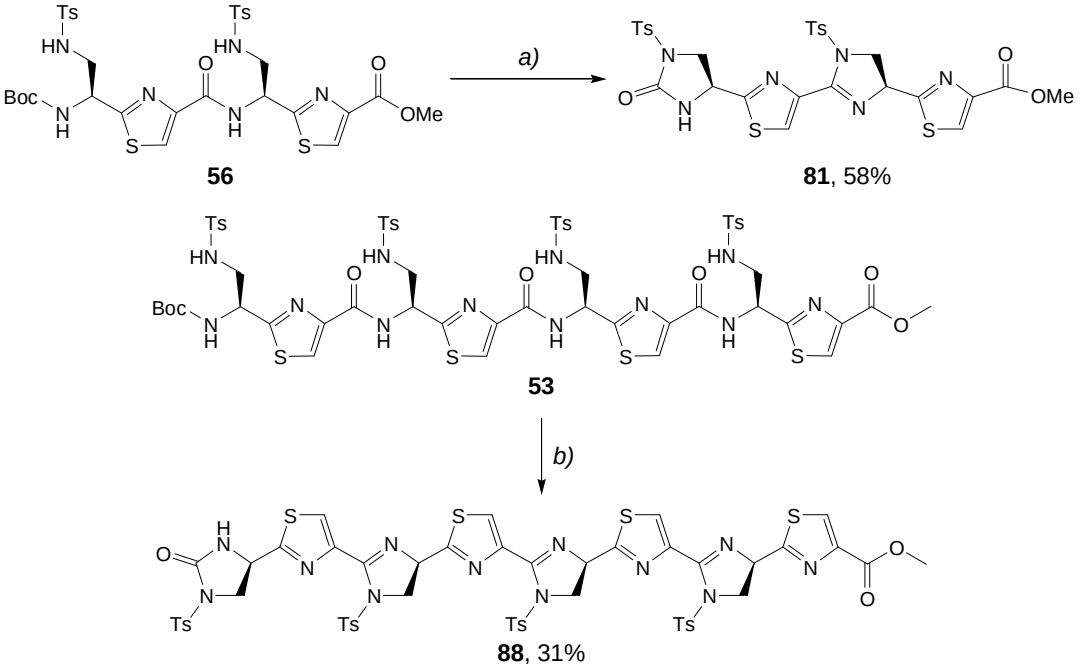
e. Using phosphoanhydrides for amide activation

A new method for imidazoline synthesis is the procedure described by Jeffery W. Kelly in 2004[182, 197]. Kelly makes use of a phosophoanhydride first proposed by Hendrickson in 1987 when he was looking for alternatives to Mitsunobu conditions[198-201]. The phosphoanhydride is produced by reacting two equivalents of a phosphine oxide with one equivalent of trifluoromethane sulfonic anhydride to furnish the triflate salt of the phosphoanhydride (*Scheme 5.17*). A corresponding intramolecular phosphoanhydride **87** was reported by Loughlin to be a more stable but equally reactive species[202].

Scheme 5.17: *Hendrickson-type phosphoanhydrides used for cyclodehydration*



Scheme 5.18. *Kelly reactions on dimer **56** and tetramer **53***



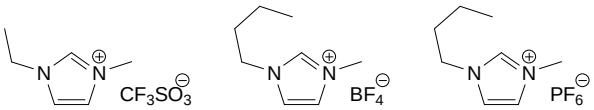
a) 6 equiv. Ph_3PO, 3 equiv. Tf_2O, CH_2Cl_2, 0°C to rt, Ar;
b) 12 equiv. Ph_3PO, 6 equiv. Tf_2O, CH_2Cl_2, 0°C to rt, Ar, 2.5 h.

Kelly used these phosphoanhydrides that were produced *in situ* to conduct cyclodehydrations with β-(tosylamino)amides. This procedure was tested on the

dimer **56** according to the published reaction conditions[182, 197]. Using CH_2Cl_2 as solvent, the conversion to a new product was quite fast, but the yield was only around 50%. The isolated product was found to be the urea derivative **81**. The same reaction was attempted with the tetramer **53**, giving the analogous product **88** in about 30% yield (*Scheme 5.18*).

Initial experiments using the Kelly reaction and macrocycle **58** showed no consumption of starting material due to the poor solubility of compound **58** in CH_2Cl_2. Different solvents were therefore tested with model compound **66**. Both Ph_3PO and **87**[197, 202] were used for phosphoanhydride formation. The phosphoanhydrides require very dry solvents and do not tolerate nitriles or ethers. Using pyridine leads to decomposition of **66**. The same was observed for a 1:1 mixture of $CHCl_3$ and Tf_2O. Pure $CHCl_3$ worked well, but was not polar enough to dissolve **58**. We then investigated the use of highly polar, but non-coordinating ionic liquids. Since these solvents tend to be hygroscopic, they first were treated with Tf_2O. The resulting TfOH and excess of Tf_2O were removed *in vacuo*, and the β-(tosylamino)amide was added. Experiments with different ionic liquids (*Figure 5.6*) gave only partial conversion of **66**. In the case of the macrocycle, **58**, the desired product was not observed. There was no relevant difference observed between phosponium anhydrides derived from Ph_3PO and **87** as they behaved quite comparably in all these reactions.

Figure 5.6: *The ionic liquids: 3-ethyl-1-mehtylimidazolium trifluoromethanesulfonate, 3-butyl-1-methylimidazolium tetrafluoroborate and hexafluorophosphate*



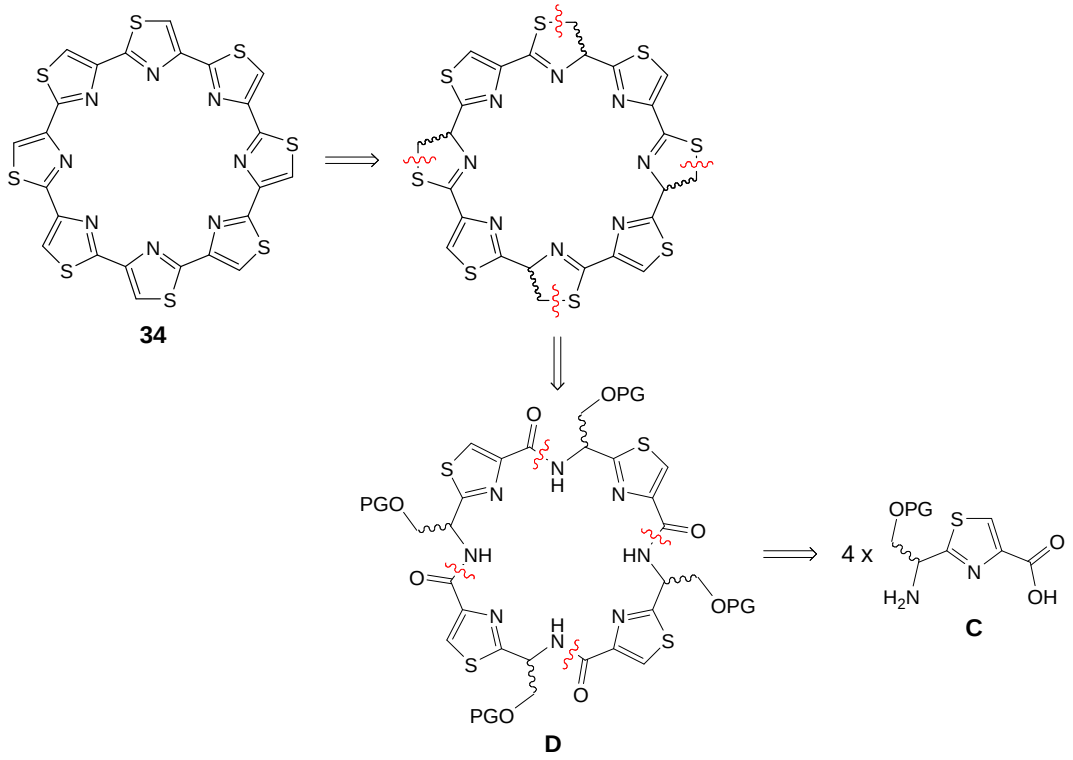
Unfortunately, **62** could not be produced applying the Kelly conditions. It is not known if the reaction is inhibited by the poor solubility of the macrocycle or if the phosphoanhydride is too reactive and decomposes faster than the reaction occurs. Based on the findings with the test substrate, **62** is even more polar than **58**. This may further complicate the completion of four ring-closures as each unit formed may further decrease solubility.

5.2 Target 2: [0.$_8$](2,4)1,3-Thiazolophan (34)

5.2.1 Retrosynthetic analysis

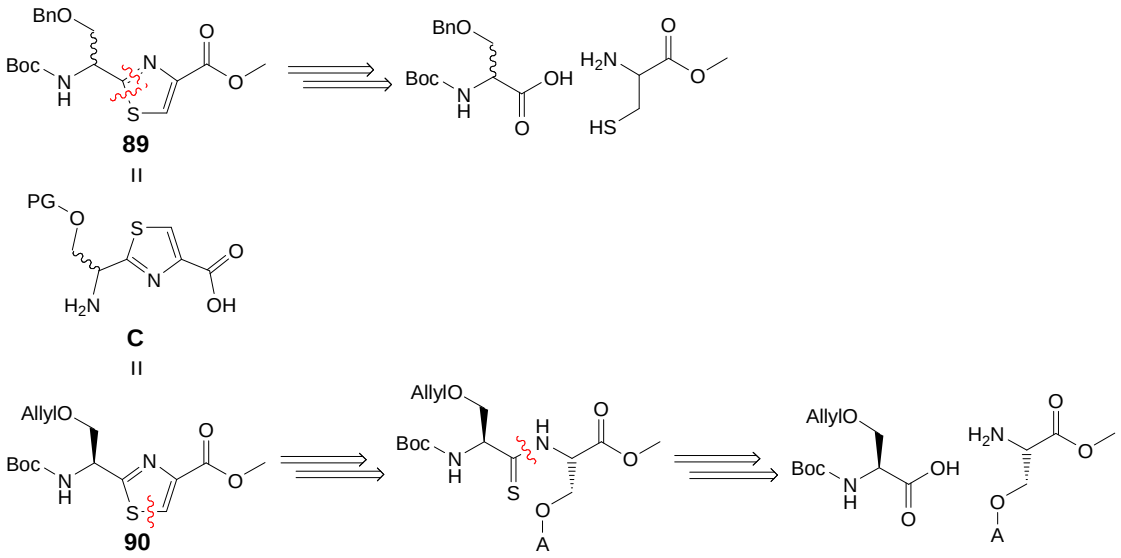
[0.$_8$](2,4)1,3-Thiazolophan (8TP, **34**) is expected to be distorted from planarity, and may therefore exhibit enhanced solubility properties as compared to planar analogs. Thanks to the high symmetry of **34**, this compound can be derived from four identical thiazole-containing amino acids. A suitable building block type "**C**" contains a protected serine side chain which gives the possibility to form thiazole units upon thionation and cyclodehydration with the amide groups (*Scheme 5.19*). Macrocycle type "**D**" is also a possible starting point for target molecule **33**, as we saw in the last chapter. This makes building block type "**C**" and macrocycle type "**D**" very valuable intermediates.

Scheme 5.19. *Retrosyntesis of macrocycle **34***



Thiazole amino acids of type "**C**" can be synthesised by fusing an activated *N*- and *O*-protected serine with a cysteine to provide a thiazoline that can be oxidized to the thiazole **89** (*Scheme 5.20*). Alternatively, allyl protected **90** can be obtained by thionation of a fully protected diserine followed by nucleophilic attack of the thioamide sulphur to the activated serine side chain (*Scheme 5.20*).

Scheme 5.20. *Retrosynthesis of building block type "**C**"*



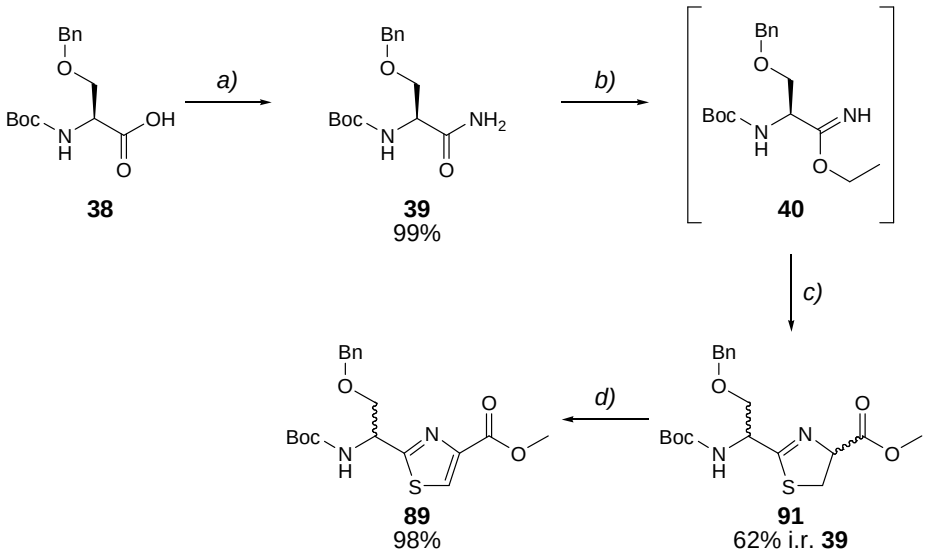
5.2.2 Synthesis of thiazole-containing amino acids

5.2.2.1 Synthesis of Boc-TSer(OBn)-OMe (**89**)

To synthesize methyl 2-{(*rac*)-2-(benzyloxy)-1-[(*tert*-butoxycarbonyl)amino]ethyl}-1,3-thiazole-4-carboxylate (Boc-TSer(OBn)-OMe, **89**) the activated acid carbon in *O*-ethyl (2*S*)-3-benzyloxy-2-[(*tert*-butoxycarbonyl)amino]propanimidoate (**40**) was reacted with H-Cys-OMe.HCl under basic conditions. The resulting thiazoline was then oxidized using DBU and $BrCCl_3$[182, 203]. This procedure is highly efficient as it provides compound **89** over four steps in 61% total yield (*Scheme 5.21*). The critical step is the fusion of the two amino acids to form thiazoline **91**. This reaction is amenable to scale-up. Optimized procedures for the preparation of 75 g of **91** in one batch were established. The structure of **89** was confirmed with x-ray crystallography (*Figure 5.7*).

This ring-closure stratety is convenient and high yielding, but the basic reaction conditions cause racemization of the stereogenic centers (*Scheme 5.21*), and lead to a mixture of diasteroisomers for **91** and pair of enantiomers for **89**. This should not pose a serious problem as all stereogenic centers are lost during formation of the final products **32**, **33** and **34** (*Scheme 5.7*).

Scheme 5.21. *Synthesis thiazole-containing amino acid* ***89***



a) 1.5 equiv. Boc_2O, 0.66 equiv. pyridine, 1.25 equiv. $[NH_4][HCO_3]$, dioxane, rt, N_2, 16 h; b) 1.1 equiv. Et_3OBF_4, 3 equiv. $CaCO_3$, CH_2Cl_2, rt, Ar, 9 h; c) 1.1 equiv. Cys-OMe.HCl, 1 equiv. Et_3N, CH_2Cl_2, rt, N_2, 16 h; d) 2 equiv. DBU, 1.05 equiv. $BrCCl_3$, CH_2Cl_2, 0℃ to rt, 1.5 h.

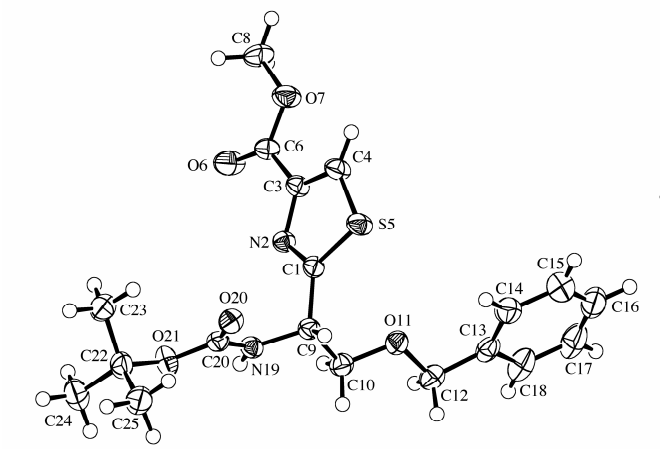


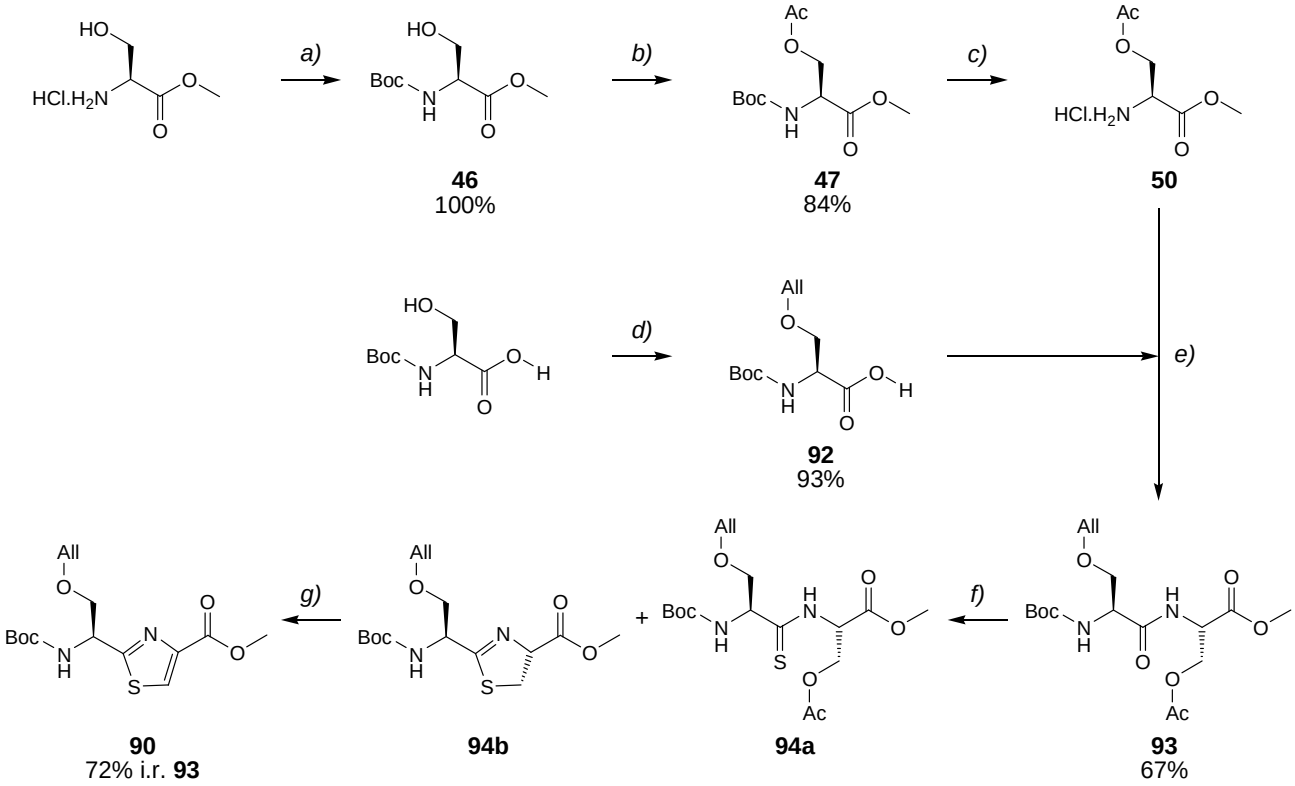
Figure 5.7: *ORTEP Plot of the molecular structure of* rac-***89*** (*50% probability ellipsoids)*

5.2.2.2 Synthesis of L-Boc-TSer(OAll)-OMe (**90**)

To produce an enatiomerically pure homolog of **89**, the synthesis of methyl 2-{2*S*-2-(benzyloxy)-1-[(*tert*-butoxycarbonyl)amino]ethyl}-thiazole-4-carboxylate (L-Boc-TSer(OAll)-OMe, **90**) was performed. Serine was protected as either Boc-Ser(OAc)-OMe (**47**) or Boc-Ser(OAll)-OH (**92**). Mild coupling conditions using TBTU and NMM in CH_2Cl_2 resulted in retention of configuration in each product. The resulting Boc-Ser(OAll)-Ser(OAc)-OMe (**93**) was treated with Lawesson's reagent in boiling toluene. Using precisely 0.5 equiv. of the reagent provides the thioamide **94**[185] without affecting the carbonyl groups in the ester or carbamate groups. Typical

reaction times are two to three hours. Longer reaction times result in the formation of thiazoline **94b** by elimination of acetic acid. A mixture of **94a** and **94b** can be carried forward directly into the next step. By taking 3.5 equiv. DBU and 1.2 equiv. $BrCCl_3$ the thioamid **94a** and thiazoline **94b** are converted directly into thiazole **90** in 72% over two steps (*Scheme 5.22*). This type of ring-closure strategy will also be used in the final steps of the ultimate target molecules (**32**, **33**, **34,** *Scheme 5.7*).

Scheme 5.22. *Synthesis of the thiazole-containing amino acid **90***



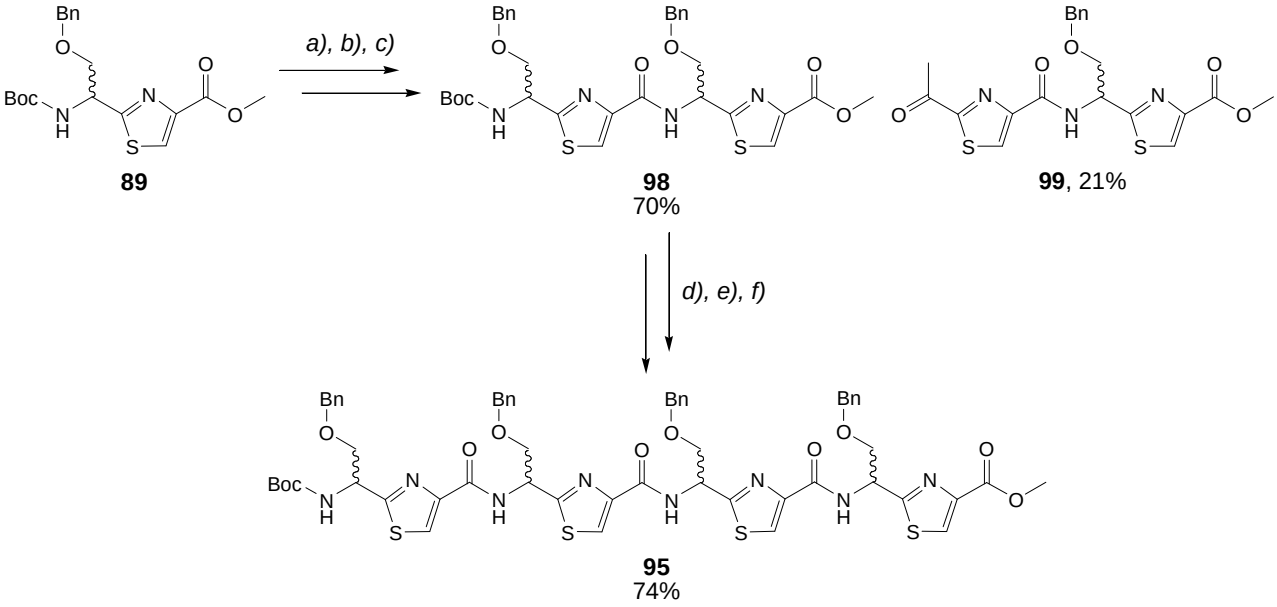
a) 1.1 equiv. Boc_2O, 2.2 equiv. Et_3N, dry CH_2Cl_2, 0° to rt, N_2, 3 h; b) 1.5 equiv. Ac_2O, 2 equiv. Et_3N, 3 mol% DMAP, CH_2Cl_2, 0℃ to rt, 1.5 h; c) TFA/CH_2Cl_2 1:3, rt, N_2; d) 2.5 equiv. NaH, 1 equiv. AllBr, dry DMF, 0℃ to rt, 16 h; e) 1 equiv. TBTU, 4 equiv. NM M, CH_2Cl_2, rt, N_2, 5 h; f) 0.5 equiv. Lawesson reagent, toluene, reflux, N_2, 2 h; g) 3.5 equiv. DBU, 1.2 equiv. $BrCCl_3$, CH_2Cl_2, rt, N_2, 3 h.

The overall yield of the seven step synthesisof enantiomerically pure L-Boc-TSer(OAll)-OMe (**90**) is 40%. Building block **89** is produced as racemate, yielding 60% in four synthetic steps. As stated before, it is not necessarily a drawback to work with racemate intermediates since all stereogenic centers will be lost in the last steps of the total synthesis. For solubility reasons, a mixture of stereoisomers can even be advantageous. For macrocycle synthesis, building block **89** was therefore selected.

5.2.3 Synthesis of Boc-[TSer(OBn)]$_4$-OMe (**95**)

The amino acid **89** was selectively deprotected using either LiOH in MeOH/H_2O or TFA in CH_2Cl_2. The resulting free acid **96** and amine **97** were coupled to form the dimer **98** using either DCC, PyBroP or TBTU as a coupling agent (*Scheme 5.23*). For optimal yields and purification reasons (70% isolated yields) TBTU was selected for scale up of the reaction. An interesting side product, ketone **99**, was also isolated. This type of product was not observed in previous reactions, and the mechanism of its formation is unknown. We speculate that the pathway to **99** involves elimination of HOBn leading to an enamine. Its tautomer, the imine, is then hydrolysed to release product **99** (*Scheme 5.24*). Even though **99** was initially an unwanted product, it turned out to be a useful test substrate for macrocycle **60**. More details on these reactions will be given in sections 5.2.7 and 5.3.3.

Scheme 5.23. *Synthesis of the tetramer **95***

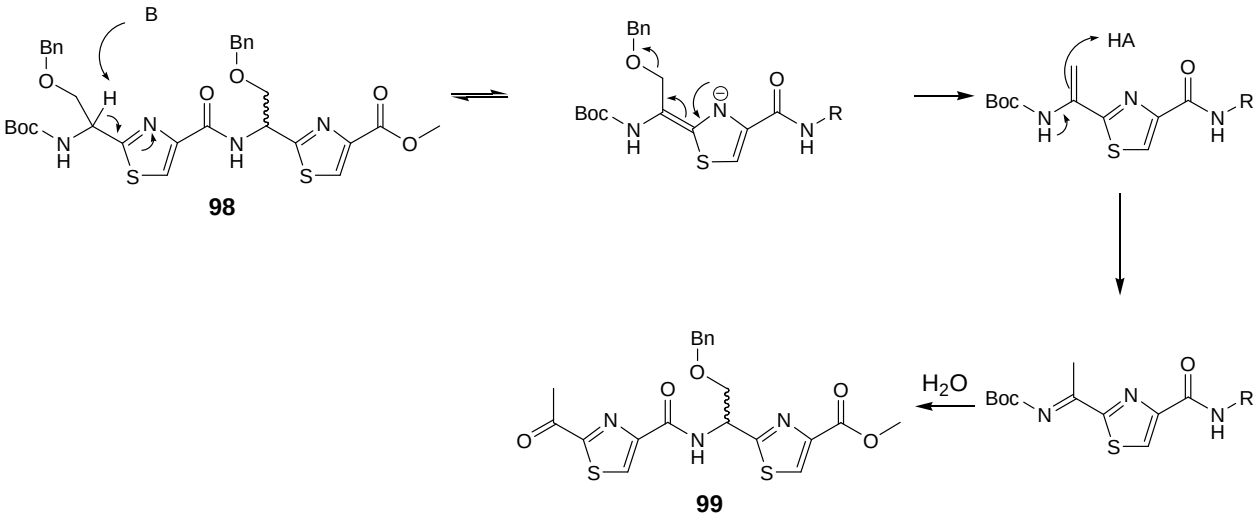


a) 6 equiv. LiOH, MeOH/H_2O 1:2, rt, N_2; b) TFA/CH_2Cl_2 1:3, rt, N_2; c) 1 equiv. TBTU, 5 equiv. NMM, rt, N_2, 16 h; d) 6 equiv. LiOH, MeOH/H_2O 1:2, rt, N_2; e) TFA/CH_2Cl_2 1:3, rt, N_2; f) 1.5 equiv. PyBOP, 6 equiv. NMM, dry DMF, rt, N_2, 16 h.

The second dimerization of **98** to compound **95** was first performed under the same reaction conditions using 1 equiv. TBTU in CH_2Cl_2 with 5 equiv. Et_3N or NMM at room temperature. Upon scale up of this reaction, the yield dropped below 50%. Taking dry DMF with NMM and 1.5 equiv. of PyBOP as coupling reagent increased the yield to 65% (*Scheme 5.23*). With the tetramer **95**, solubility started to become an

issue. The molecule is still somewhat soluble in $CHCl_3$, but line broadening and splitting in ^{1}H-NMR spectra suggest the formation of secondary structures.

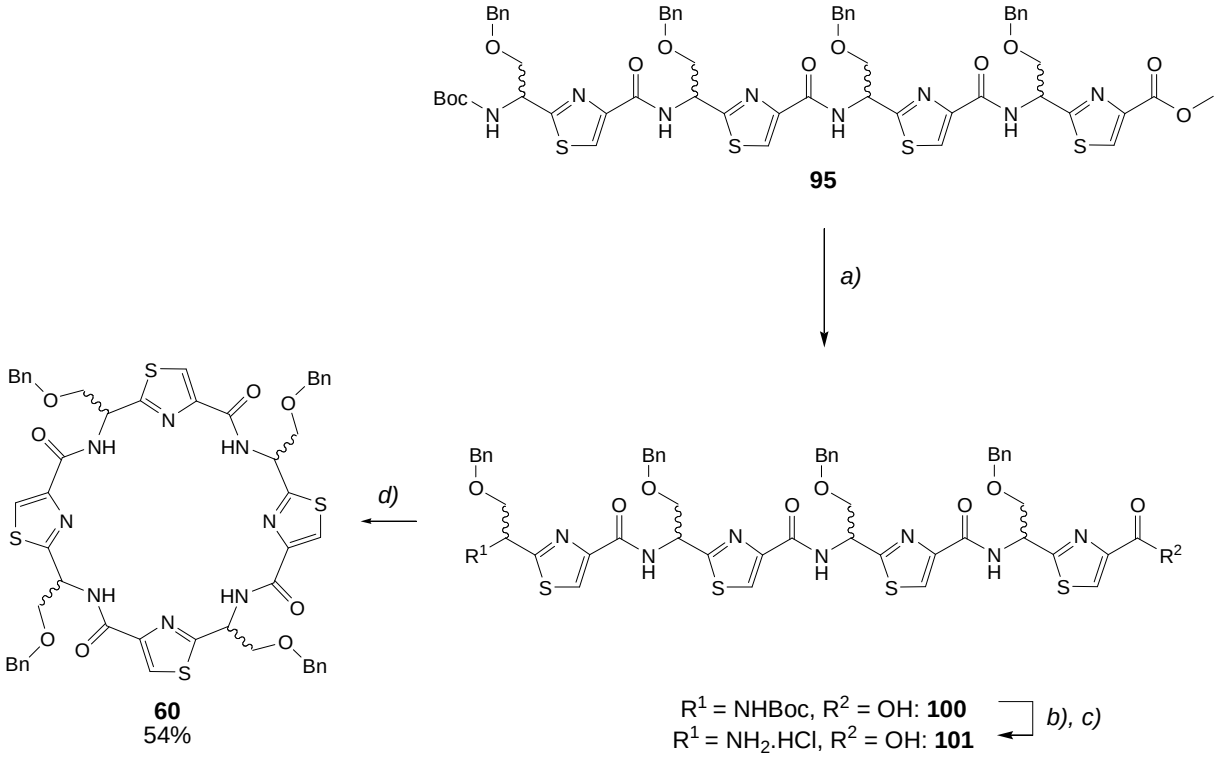
Scheme 5.24. *Proposed mechanism for formation of side product **99***



5.2.4 Macrolactamization

The limited solubility of **95** necessitated the use of HPLC to monitor the progress of the deprotection reactions for the free acid **100** and the amino acid **101** (*Scheme 5.25*). For optimal deprotection of **95**, the concentration of starting material was decreased below 5 mM in a 6:1 mixture of MeOH and H_2O. For subsequent Boc deprotection, CH_2Cl_2 and TFA were used in a ratio of 8:1. This reaction could be performed at higher concentrations of about 20 mM due to the solubilizing effects of TFA. The TFA salt was converted to the HCl salt using Dowex ion exchange resin. For macrolactamization, a 1.5 mM solution of **101** in DMF was reacted for 30 h with 5 equiv. PyBOP and 10 equiv. NMM in the dark at rt under N_2. The reaction was again monitored by HPLC. Under these conditions, yields up to 65% for the conversion of **95** to **60** could be achieved (*Scheme 5.23*).

Scheme 5.25. *Formation of macrocycle* ***60***



a) 5 equiv. LiOH, MeOH/H_2O 1:3, rt, N_2; b) TFA/CH_2Cl_2 1:1, rt, N_2; c) Anion exchange: Dowex, MeOH, rt, 16 h; d) 5 equiv. PyBOP, 10 equiv. NMM, dry DMF (c = 1.5 mM), rt, N_2.

The ^{1}H-NMR of macrocycle **60** is consistent with a mixture of diastereoisomers (*Figure 5.8*). A set of seven signals for the thiazole-H between 8.4 to 8.2 ppm and the benzyl aromatic CH between 7.3 and 7.1 ppm were observed.

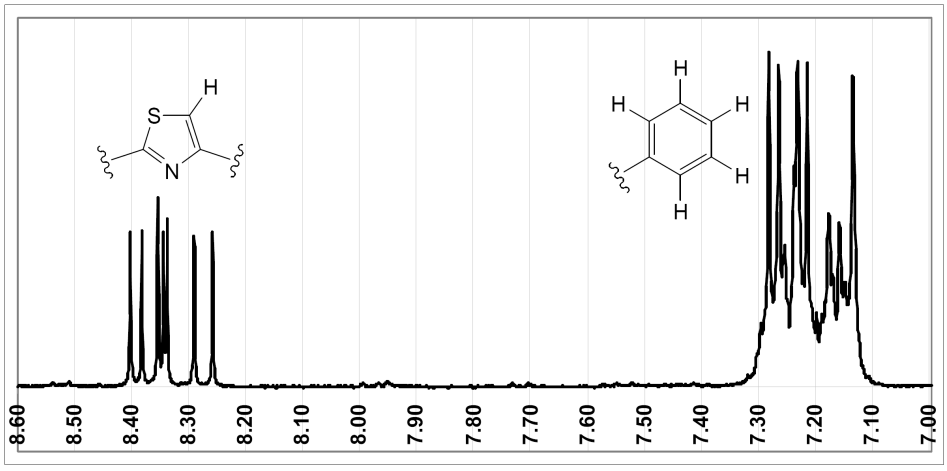


Figure 5.8: *^{1}H-NMR of the mixture of diastereoisomers of* ***60*** *in DMSO. NH groups are not visible since a drop of MeOD was added.*

The set of seven signals can be explained by the presence of four possible diasteroisomers and corresponding enantiomers: *RRRR*/*SSSS*, *RSRR*/*SRSS*,

RSRS/*SRSR* and *RRSS*/*SSRR* (*Figure 5.9*). It is likely that some of the thiazole proton signals are overlapping, but there should theoretically be one resonance for the *RRRR* (C_4 symmetry) diasteroisomer, four for the *RSRR* (C_1), two for *RSRS* (C_2) and four for *RRSS* (C_1), giving eleven signals all together.

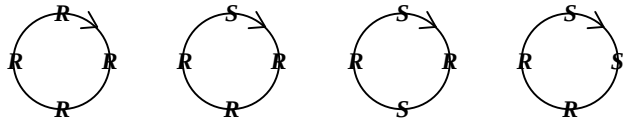


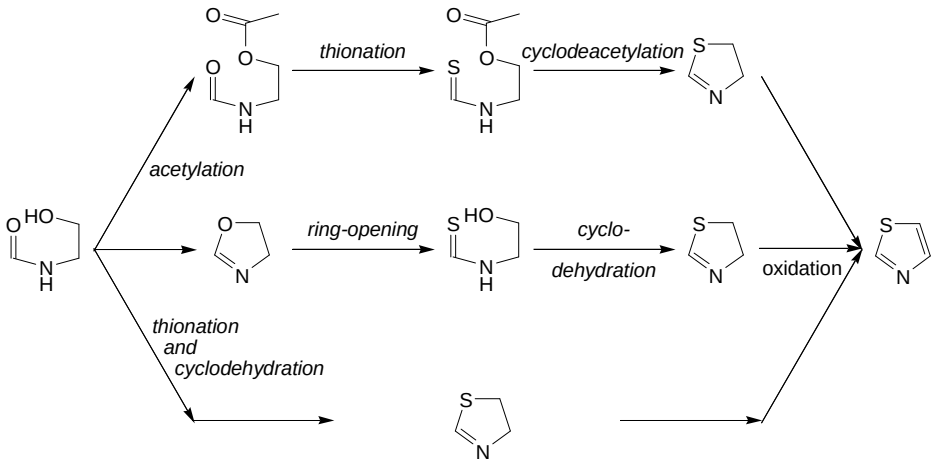
Figure 5.9: *Possible diastereoisomers of macrocycle* ***60***

Following purification, macrocycle **60** exhibited poor solubility properties in solvents like AcOEt, MeCN, and $CHCl_3$, but partial solubility in mixtures of $CHCl_3$ or AcOEt and MeOH. Very powerful solvents like DMF, DMSO or TFA were necessary to fully dissolve **60**.

Compound mixture **60** exhibits a R_f value of 0.8 in $CHCl_3$:MeOH 10:1. It is less polar than compound **58**, that exhibits a R_f value of 0.25 in $CHCl_3$:MeOH 10:1. In addition to differencies in apparent polarity, the mixture of diasteroisomers in **60** also increases the solubility of the macrocycle.

5.2.5 Strategies for formation of thiazole units

Scheme 5.26 *Strategies towards the thiazole*



On the basis of the tetrabenzylmacrocycle **60**, three possible pathways to the target molecule 8TP (**34**) were considered (*Scheme 5.26*). The first is the conversion of the β-acetoxyamide to the β-acetoxythioamide, followed by intramolecular substitution of

the acetoxy group to form the thiazoline. The second is the ring-opening of oxazole with sulphur, followed by cyclodehydration. The third path is substitution of one of the oxygen atoms by sulphur followed by cyclodehydration, where P_2S_5 serves as both thionating and dehydrating agent.

Five model compounds were used to test these potential pathways (*Figure 5.10*). **63** is the most robust compound having few reactive functional groups and no sulphur in the compound. The test substrate most similar to macrocycle **60** is compound **99**. The core is an exact copy of the macrocycle, though without the expected ring strain and formation of secondary structures. However, unlike macrocycle **60**, the methylketone of **99** tends to enolize upon deprotonation and can lead to irrelevant side reactions.

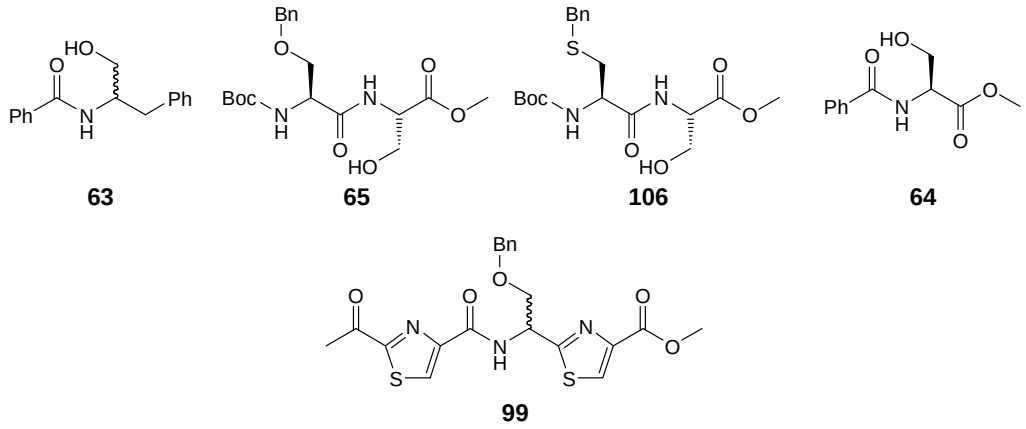
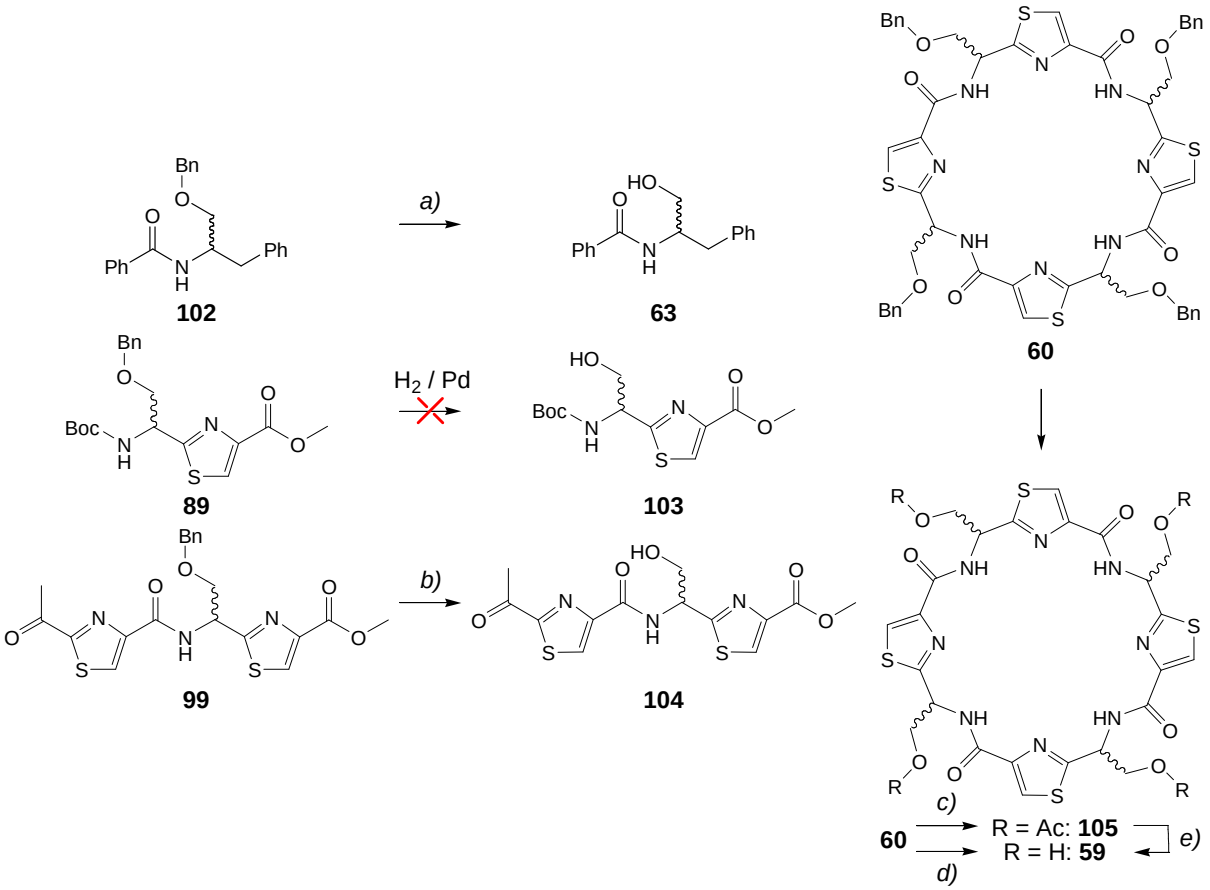


Figure 5.10: *Model compounds for thiazole formation*

5.2.6 Debenzylation of the alcohols

To convert β-hydroxyamides to thiazoles or oxazoles the "serine" alcohols of macrocycle **60** were first deprotected. Catalytic hydrogenation of macrocycle **60** using 10% Pd on activated charcoal in MeOH or AcOEt failed to work for both **60** and the test substrate **89**. Elevated temperatures, AcOH and H_2 at 45 bar did not improve this result. Transhydrogenation with cyclohexene and $Pd(OH)_2$ in EtOH[204] also did not work. Apparently, the thiazole sulphur poisons the Pd catalyst.

Scheme 5.27. *Deprotection of the benzyl protecting group*



a) 7.5 equiv. anisole, 6 equiv. $AlCl_3$, CH_2Cl_2, rt, N_2, 16 h; b) 11 equiv. Tf_2O, 10 equiv. $BF_3.Et_2O$, CH_2Cl_2, 0℃ to rt, N_2, 16 h; c) 40 equiv. $BF_3.Et_2O$, CH_2Cl_2:Ac_2O 1:1, rt, N_2, 16 h; d) 40 equiv. Tf_2O, 36 equiv. $BF_3.Et_2O$, $ClCH_2CH_2Cl$, rt, N_2, 4 h; e) 10 equiv. LiOH, MeCN:MeOH:H_2O 1:1:1, 45℃, 8 min.

Alternative procedures for benzyl removal were evaluated. Test reactions were first performed on simple model compounds **102**, **89** and **99**[1] (*Scheme 5.27*). **102** could be deprotected upon treatment with Raney-Nickel in a mixture of MeOH and H_2O under 1 atm of H_2. Analogous treatment of **89** did not give the desired product, but instead, the reduction of the thiazole to the thiazoline compound **91** was observed. **102** could also be deprotected using a mixture of $PhI(OAc)_2$, $TsNH_2$, tin powder, Al_2O_3 and $Cu(CF_3SO_3)_2$ according to He[205]. The analogous experiment with **89** gave no desired product. Deprotection of **89** and **99** to furnish **103** and **104** was accomplished using anisole and $AlCl_3$ in CH_2Cl_2[206]. This reaction was attempted in more polar solvents like $CHCl_3$, DME, MeCN and DMF. Only $CHCl_3$ and CH_2Cl_2 were found to be compatible. Deprotection of macrocycle **60** using anisole in CH_2Cl_2 resulted in partial conversion, where one or two of the four benzyl groups could be removed. To optimize this reaction, $BF_3.Et_2O$ was used as the Lewis acid in a mixture

[1] Some of these reactions were evaluated by Renate Brokamp, a student doing her advanced practicum in our lab.

of Ac_2O and CH_2Cl_2[207]. This process delivers not directly the alcohol but rather the tetraacetate macrocycle **105** (*Scheme 5.27*) that could be isolated in yields of up to 60-70%. The best yields for hydrolysis of the tetraacetate were obtained by suspending **105** in a 40°C mixture of MeCN, MeOH, and H_2O (4:4:1), and adding 10 equiv. of LiOH in H_2O to the suspension. After 5 to 6 min at 40°C, 10 equiv. of HCl were added to neutralize the reaction. This procedure yielded 60-80% of the tetraalcohol **59** after column chromatography.

Having developed this procedure, we speculated that other anhydrides might be used to convert the benzyl group into an activated alcohol for subsequent ring closure. Deprotection with $BF_3.Et_2O$ and $(CF_3CO)_2O$, $(CF_3SO_2)_2O$, $(CH_3SO_2)_2O$, $(C_7H_7SO_2)_2O$ and $(C_9H_{11}SO_2)_2O$ failed to give clean conversions to the desired products. With the trifluoroacetates and trifluoromethanesulfonyl groups, the tetraalcohol **59** could be isolated in moderate yields following aqueous workup of the reaction. This type of deprotection was optimised by replacing CH_2Cl_2 with $ClCH_2CH_2Cl$ to give maximal yields of 60-65%. While this one step deprotection reaction was time saving, purification of the tetraalcohol **59** on SiO_2 was much more challenging and less amenable to scale-up as compared to tetraacetate **105**.

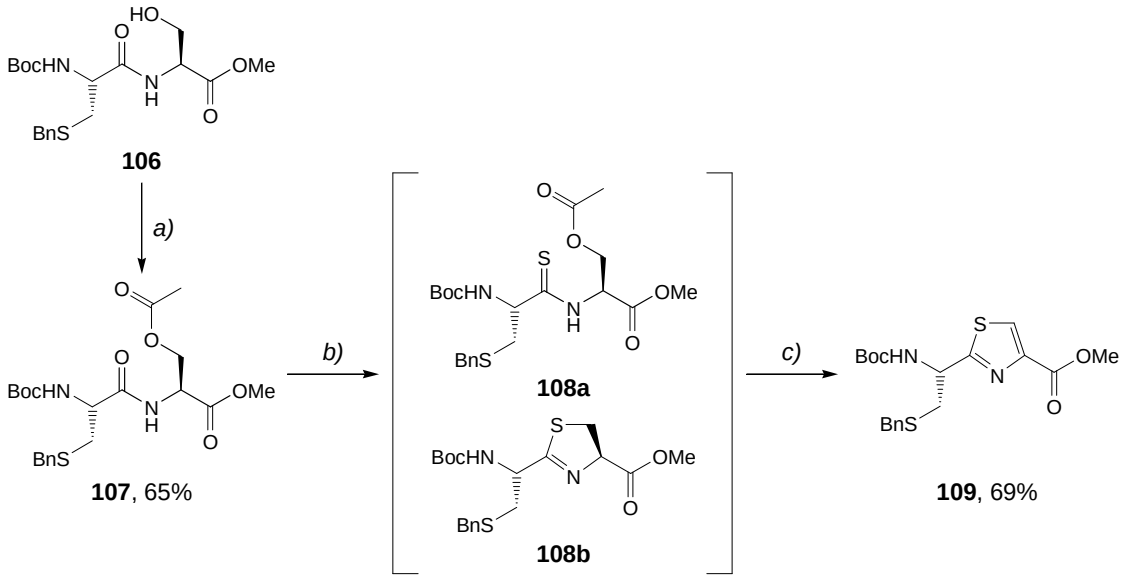
5.2.7 Results and discussion

5.2.7.1 Reactions using β-acetoxyamides

It is known that β-acetoxyamides form thiazolines upon refluxing with Lawesson reagent in toluene[185] (e.g. in *Scheme 5.28* with **106**, **107**, **108** and **109**). This approach was used for the synthesis of building blocks **44** and **90** (chapter 5.1.2.2 and 5.2.2.2). Thionation and thiazoline ring-closure were attempted with macromolecule **105**. Using 0.5 equiv. of Lawesson's reagent in refluxing toluene resulted in only partial conversion. Adding more Lawesson's reagent did not push the reaction to completion. Most of the recovered material was, according to ESI-MS, the starting material with one oxygen exchanged by sulphur ($[M_{\mathbf{105}} - O + S + Na]^+$). There were also traces of a single-thiazoline containing macrocycle ($[M_{\mathbf{105}} - O + S - HOAc + Na]^+$) and a single-thiazoline containing ring with a second oxygen exchanged by sulphur ($[M_{\mathbf{105}} - 2\ O + 2\ S - HOAc + Na]^+$). The lack of reactivity was ascribed to the poor solubility of **105** in toluene. Other solvents were tried, but no

product could be detected using benzonitrile and P_2S_5, or using ethers solvents[208] containing chaotropic salts like LiCl or LiOTf.

Scheme 5.28. *Formation of thioazoles using β-acetoxyamides*



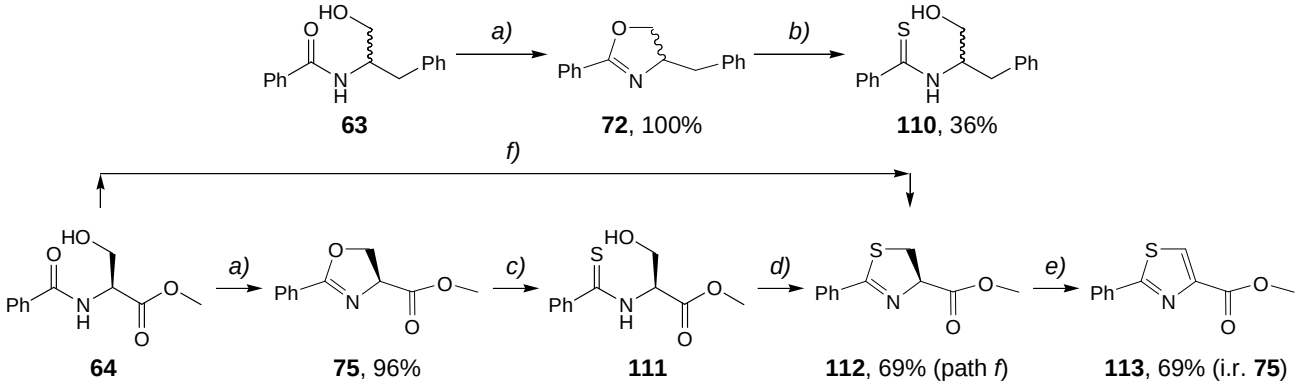
a) 2.5 equiv. Ac_2O, pyridine, 0℃ to rt, N_2; b) 0.5 equiv. Lawesson's reagent, toluene, reflux, N_2, 2 h; c) 2 equiv. DBU, 1.05 equiv. $BrCCl_3$, CH_2Cl_2, rt, N_2, 1 h.

5.2.7.2 Oxazolines as intermediates towards thiazolines

Published procedures for the conversion of oxazolines into thiazolines involve exchanging the oxygen by sulphur in a one pot reaction, or opening the oxazoline with sulphur to give a β-hydroxythioamide followed by ring-closure[209]. Experiments using test substrates were used to evaluate the feasibility of these approaches. Oxazoline **72** was treated with a mixture of Et_3N, AcOH and Na_2S x 9 H_2O in MeOH (*Scheme 5.29*). The reaction was not complete after 16 h, so the mixture was heated at 60℃ for 6 h. 59% of the oxazoline was isolated again and only 36% of the desired product **110** was isolated. The thioamide carbon of **110** exhibits a downfield resonance at 199 ppm as compared to 168 ppm for **63**.

A slightly different procedure was used for oxazoline **75**[210, 211]. **75** was dissolved in a 1:1 mixture of MeOH and Et_3N, the solution was purged with H_2S for 20 min and stirred at rt for 16 h. TLC indicated complete conversion to **111**. The isolated product **111** could be converted quantitatively to **112** using the DAST reaction, and subsequently oxidized to **113** using $BrCCl_3$ / DBU with a yield of about 70%.

Scheme 5.29. *Oxazoline opening by H_2S*



a) 1.25 equiv. DAST, CH_2Cl_2, -5°C to rt, N_2; b) 5 equiv. $Na_2S.9H_2O$, 50 equiv. Et_3N, 10 equiv. AcOH, MeOH, 0°C to rt, N_2, 4 d; c) H_2S (g), MeOH/Et_3N 1:1, rt, 2 h; d) 1.5 equiv. DAST, CH_2Cl_2, -10°C to rt; e) 2 equiv. DBU, 1.2 equiv. $BrCCl_3$, CH_2Cl_2, 0°C to rt, N_2, 2 h; f) 2 equiv. P_2S_5, pyridine, reflux, N_2, 2 h.

5.2.7.3 Thionation and dehydration of β-hydroxyamides

Xu, Du *et al.* reported the formation of thiazolines directly from β-hydroxyamides by refluxing with P_2S_5 in pyridine[212]. They used this procedure for the synthesis of 4,5-dihydrothiazoles in yields of approximately 50% for most examples given. Since the tetraalcohol **59** is highly soluble in pyridine, this approach seemed to be quite promising. This conversion was first evaluated using test compound **64** and yielded almost 70% of the desired thiazoline **112** (*Scheme 5.29*, path *f)*). Attempts to improve the yield by using Lawesson's reagent were fruitless. Jan Bergman *et al.* claimed that a faster degradation of the Lawesson's reagent is observed at elevated temperatures as compared to P_2S_5[213]. In addition, they reported a complex of pyridine and P_2S_5 as an analog of Lawesson's reagent but being more stable and reactive (*Figure 5.11*). The reactive pyridine phosphosulfide reagent **114** can be isolated and used in other solvents leading to better yields and cleaner conversions than other thionation reagents. **114** was produced according to Bergman[213] and its reaction with **64** was tested using MeCN and Me_2SO_2 as solvents. The reaction showed very clean conversion and an improved yield of 90% for the desired thiazoline. The subsequent oxidation of **112** was accomplished using 2 equiv. DBU and 1.2 equiv. $BrCCl_3$ to give **113** in 70% yield.

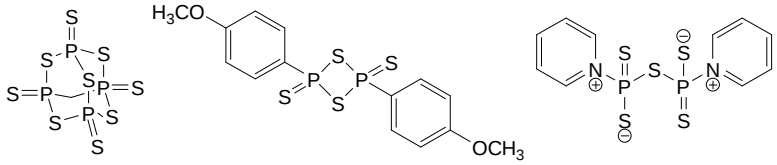
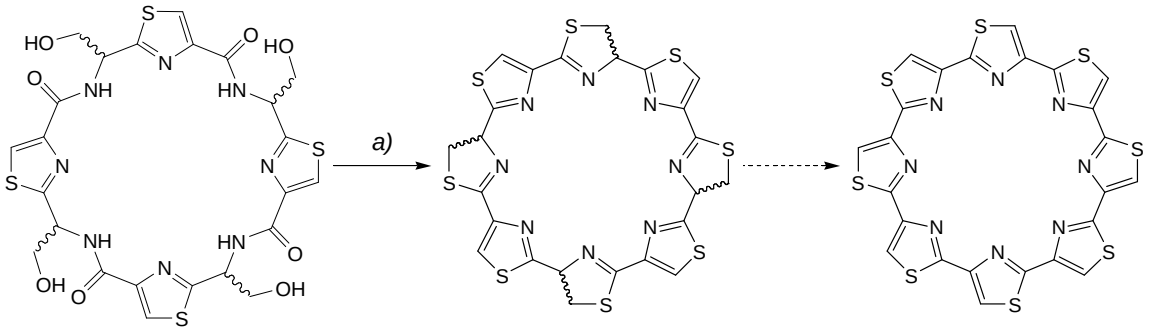


Figure 5.11: *Reagents used for thionation reactions*

Scheme 5.30. *Formation of 8TP (**34**)*



a) 8 equiv. P_2S_5, pyridine, reflux, N_2, 5 h

Macrocycle **59** was therefore treated with P_2S_5 in pyridine. The solution was refluxed for 5 h. A ladder of different products was observed. Aqueous workup was no problem in the case of the test substrates, but was avoided in the case of the macrocycle to prevent hydrolysis. The purity and yield of this reaction could be improved using reagent **114** and performing the reaction in MeCN. The resulting material **115** (*Scheme 5.30*) was subjected to oxidation – and was lost! It was dissolved in dry DMF and cooled down to 0℃ in an ice bath. Addition of DBU resulted in an immediate red colouration of the solution, possibly indicating the removal of the α proton resulting in enlarged and negatively charged π system. Addition of the $BrCCl_3$ gave a brown solution which got darker by time. ESI-MS revealed a weak signal possibly belonging to the desired product. Changing the order of addition, first adding an excess of $BrCCl_3$ present then a solution of DBU didn't improve the results.

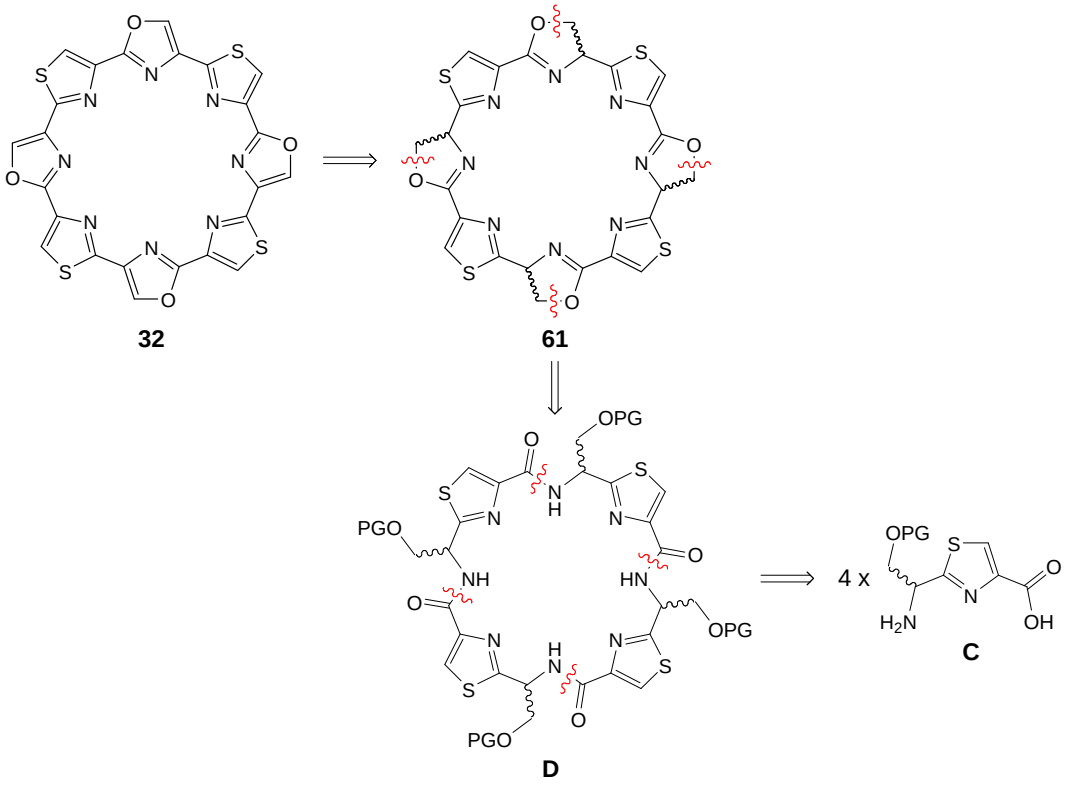
These observations lead to the crucial question of the nature of the target molecule. Does it tend to aggregate, precipitate or is it lost during isolation, or is it simply not formed because the negatively charged intermediates are too fragile or unstable?

5.3 Target 3: [0.$_4$]{(2,4)1,3-Thiazolo[0](2,4)1,3-oxazolo}phan (32)

5.3.1 Retrosynthetic analysis

Among the three proposed target molecules, [0.$_4$]{(2,4)1,3-thiazolo[0](2,4)1,3-oxazolo}phan (4TOP, **32**) is closest relative to telomestatin. According to our calculations, the ring strain in **32** should be drastically reduced compared to [0.$_8$](2,4)1,3-oxazolophan (*Table 4.2*), but still the eight rings do not completely fill 360°, so the molecule is expected to be planar.

Scheme 5.31. *Retrosynthesis of macrocycle **32***

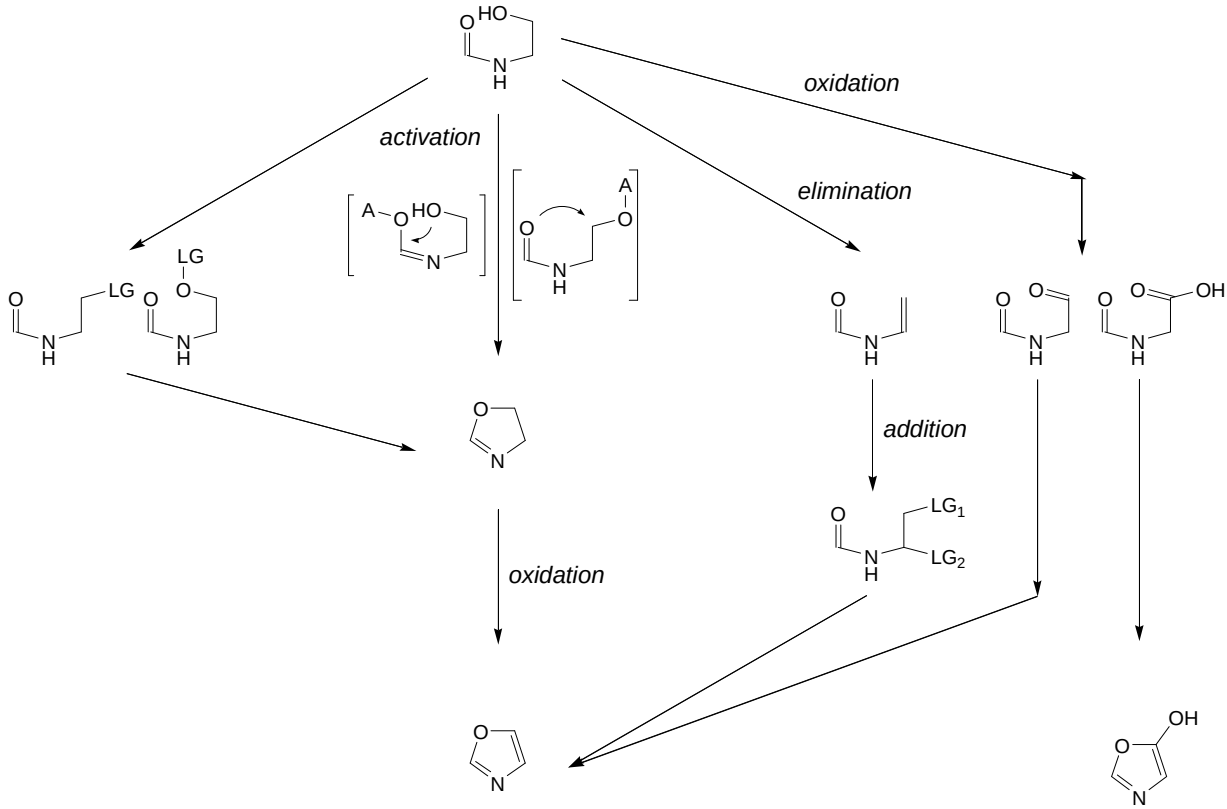


Due to the C_4 symmetry, macrocycle type "**D**" can be derived by assembling four identical thiazole-containing amino acid building blocks of type "**C**". By modifying the protected alcohol side chains on macrocycle "**D**", oxazolines are formed to furnish macrocycle **61**, which is then oxidized to the target macrocycle **32** (*Scheme 5.31*). This approach is very similar to the synthesis of 8TP (**34**, *Scheme 5.19*). The same building block **89** and the same macrocyclic precursors **60**, tetracetate **105** and tetraalcohol **59**, all described in the last chapter, could be used for the synthesis **32**.

5.3.2 Strategies for formation of oxazole units

Methods to generate an oxazole starting from a β-hydroxyalcohol include conversion of the alcohol into a good leaving group "LG" followed by substitution with the amide oxygen leading directly to the oxazoline. The same result is obtained by activation of the amide oxygen and substitution by the alcohol. The resulting oxazolines can be subsequently oxidized to the corresponding oxazoles[203, 214]. Some other strategies include elimination of the β-alcohol, addition of two potential leaving groups followed by ring-closure and elimination leading directly to the oxazole. This approach was successful in case of the total synthesis of telomestatin[157]. To avoid the oxidation step, the alcohol can be transformed into an aldehyde or acid before cyclization, leading to either an oxazole or hydroxy- or alkoxy-substituted oxazole.

Scheme 5.32. *Strategies for oxazole synthesis*



As model compounds for the macrocycle, **63**, **65**, **106**, **64**, **99** and **116** (*Figure 5.12*) were used to test ring closing reactions.

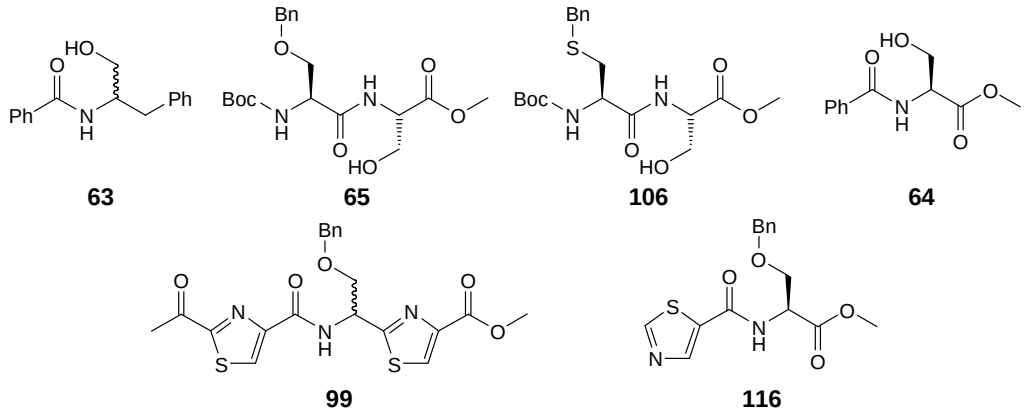
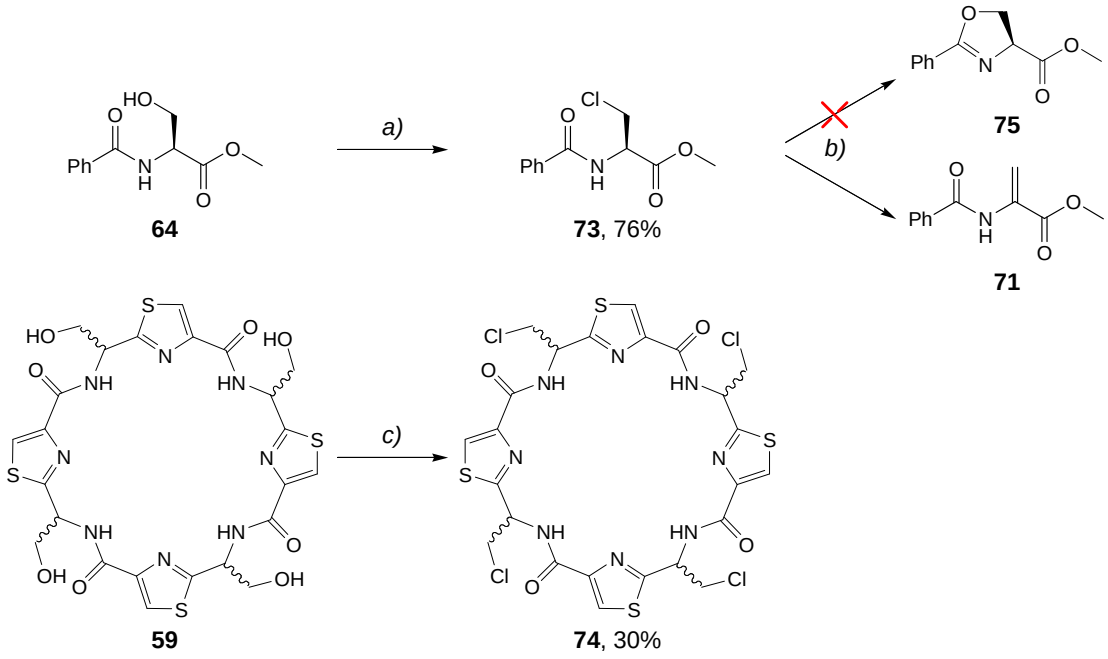


Figure 5.12: *Model substrates for oxazole synthesis*

5.3.3 Results and discussion

5.3.3.1 Substitution of OH with Cl to promote oxazoline formation

Scheme 5.33. *Monochlorination of β-hydroxyamides*



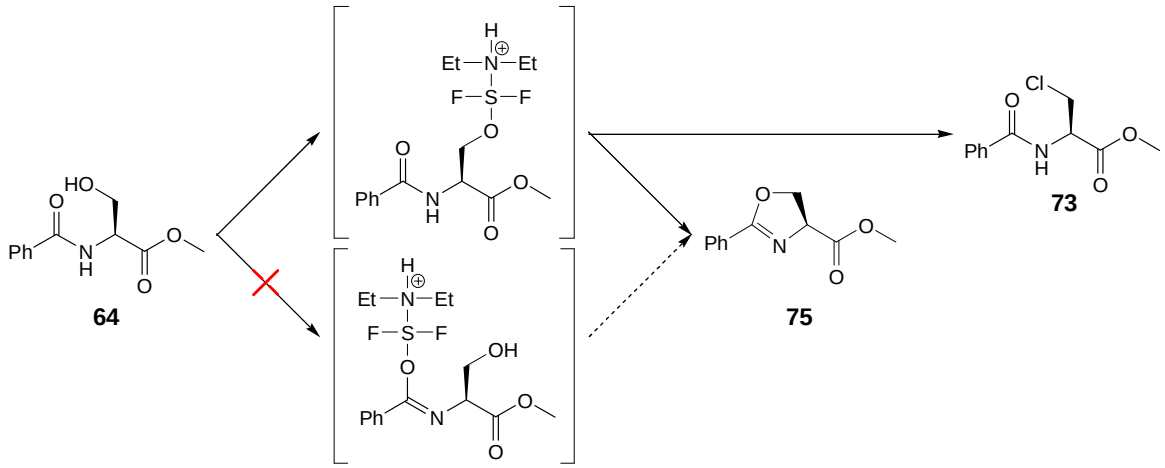
a) 2 vol% DMF, $SOCl_2$, reflux, N_2, 16 h or 1.5 equiv. DAST, 0.5 M LiCl in DMF, 0℃ to rt, N_2, b) DMAP, DMF, 0℃, N_2; c) 6 equiv. DAST, 0.5 M LiCl in DMF, 0℃ to rt, N_2.

Converting the alcohol of a β-hydroxyamide into a good leaving group is one way to promote oxazoline formation. There are many different ways to convert alcohols into chlorides. Using thionylchloride in CH_2Cl_2 or $CHCl_3$ is probably the most common one. **64** was successfully converted into β-chloroamide **73** (*Scheme 5.33*). This reaction was performed using a large excess of $SOCl_2$ in $CHCl_3$ or in neat $SOCl_2$

containing a catalytic amount of DMF. For macrocycle **59**, this reaction was not possible since **59** exhibited poor solubility in mixtures of $SOCl_2$ and $CHCl_3$.

Another method to produce the tetrachloride **74** was accidentally discovered when the solubility of **59** in THF was increased by adding LiCl as chaotropic salt. According to this procedure, model compound **64** was dissolved in a 0.5 M LiCl solution in THF. Addition of 1 equiv. DAST gives the chlorinated product **73** in 76% yield. This reaction demonstrates that DAST activates the β-hydroxyamide at the alcohol not amide (*Scheme 5.34*). Using this procedure, it might be possible to access other halogenides (Br, I) under mild and selective conditions.

Scheme 5.34. *Mechanism of the DAST activation*: *for the formation of the oxazoline activation of alcohol or amide oxygen are theoretically possible, the substitution by chloride shows that activation of the alcohol is faster.*



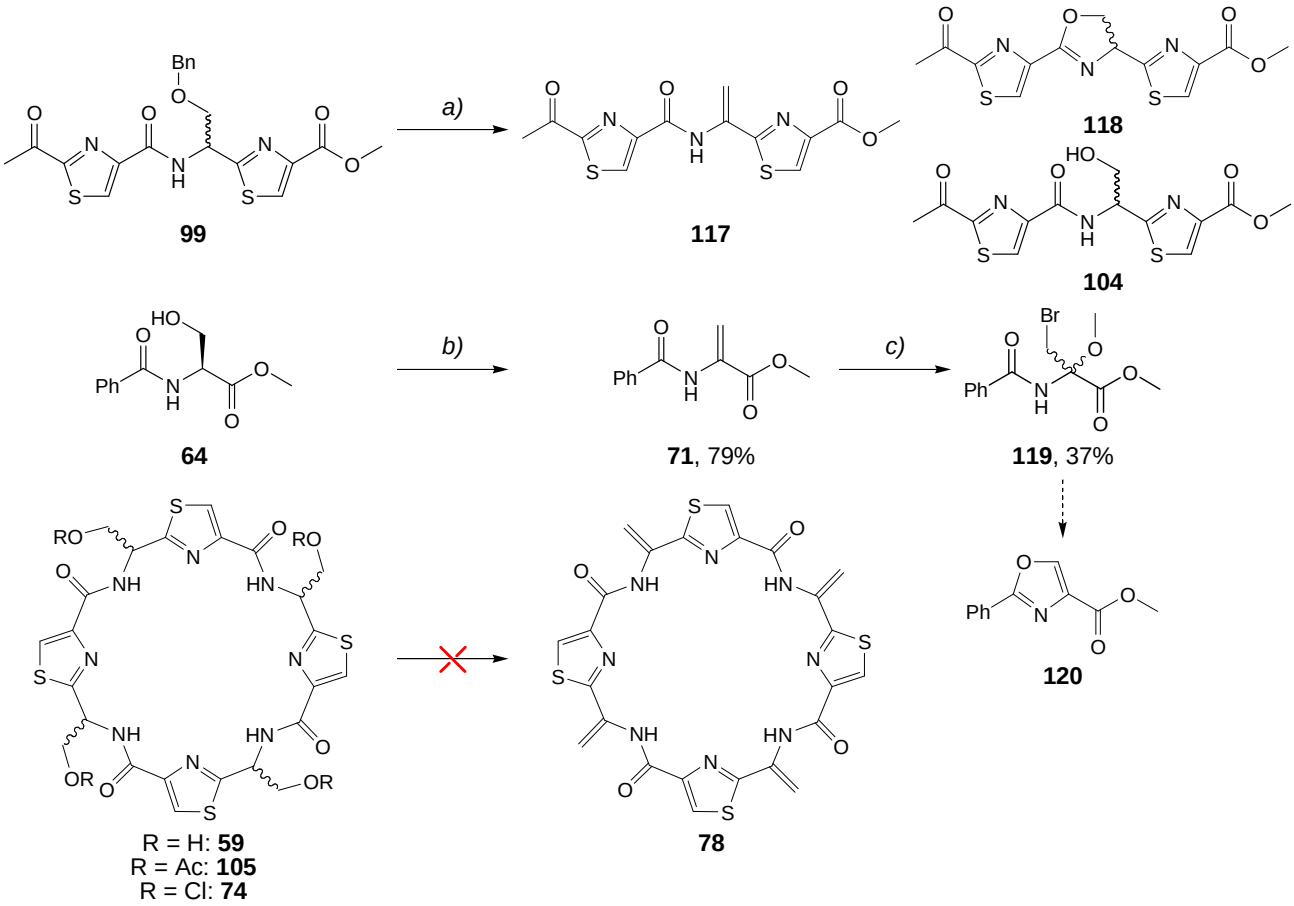
Macrocycle **59** was not soluble in a 0.5 M solution of LiCl in THF, but the chlorination reaction could also be performed using DMF as the solvent. Under these conditions, tetrachloride **74** could be isolated in 30% yield.

Cyclization of the β-chloroamides to give oxazolines was then investigated. Treatment of **73** with tertiary amines in $CHCl_3$ gave only the elimination product **71**. The same results were obtained using DMAP in DMF at 0° and with K_2CO_3 in AcOEt at 75°C. Refluxing **73** in AcOEt without base showed no consumption of starting material. Ring-closure by halogen exchange using iodide was also attempted, but conversion to the elimination product **71** was again observed. Apparently, one must remove HCl, but even mild bases cause β elimination of HCl instead of cyclization. This strategy was therefore not further developed for the macrocycle tetrachloride **74**.

5.3.3.2 Elimination of H_2O and addition of "MeOBr" to form oxazoles

As described in section 3.3.3, the method used for forming the last ring in the total synthesis of telomestatin was performed by elimination of H_2O to form a dehydroalanine unit followed by addition of Br^+ and MeO^-. We therefore investigated this pathway. A common way to facilitate elimination is to convert the alcohol into a sulfonyl group and then perform an E_2 type elimination with a sterically hindered base like DBU. In our case we aimed to combine this reaction with benzyl deprotection. A series of reactions were conducted using the model compound **99** and $BF_3.Et_2O$ as a Lewis acid, $(CF_3CO)_2O$, Tf_2O or Ms_2O as anhydride and CH_2Cl_2 or $ClCH_2CH_2Cl$ as solvents. The benzyl deprotection was followed by addition of 1 M DBU solution in DMF at 0℃ giving complex mixtures containing the d esired elimination product **117** as well as oxazoline **118** and alcohol **104** (*Scheme 5.35*). The distribution of products was evaluated using TLC, but there were no significant differences for the different solvents and anhydrides evaluated.

Scheme 5.35. *Elimination of H_2O and formal addition of BrOMe*



a) 15 vol% $(CF_3CO)_2O$, 15 equiv. $BF_3.Et_2O$, CH_2Cl_2, rt, N_2, 36 h;
b) 1.4 equiv. DPPA, 1.4 equiv. DBU, THF, 0℃ to rt, N_2, 15 h;
c) 1.05 equiv. NBS, molecular sieves (A4), CH_2Cl_2/MeOH 8:1, 0℃ to rt, N_2, 3 h.

Elimination product **71** can cleanly be synthesised by reacting alcohol **64** with DPPA followed by addition of DBU in THF as described in section 5.1.6.1 b[188]. The same reaction using **64** could also be performed in MeCN and DMF to give the elimination product **71** in yields in around 80%. The dehydroalanine product, **71**, can be converted into the bromo- and methoxy-substituted derivative **119** using NBS in a mixture of CH_2Cl_2 and MeOH at 0℃ over powdered molecular sieves[157]. Subsequent transformation into oxazole **120** was not attempted.

The elimination of four water molecules from tetraalcohol **59** using DPPA was first tried in MeCN. The mixture of products contained some of the desired material **78** according to ESI-MS. The reaction was tried again in DMF, but none of the desired material was observed, only lighter materials could be isolated, indicating decomposition of the macrocycle.

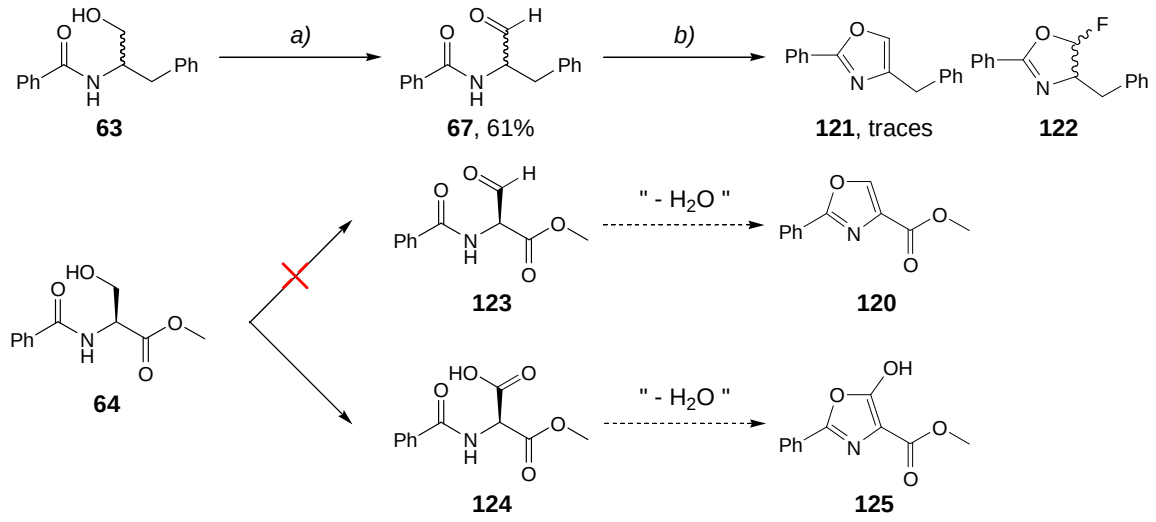
5.3.3.3 Ring-closure by Mitsunobu type reactions

Mitsunobu reactions are good candidate for this type of chemistry. Following the protocol by Oyo Mitsunobu[215] conversion of **64** to the oxazoline **75** was attempted in DME at rt using 1.5 equiv. DEAD and Ph_3P. The desired oxazoline **75** was isolated in 65% yield, and the elimination product **71** was isolated in 25 – 30% yield. Using exactly the same reaction conditions, no product could be isolated when macrocycle **59** was used as the starting material.

5.3.3.4 Oxidation to aldehyde to promote oxazole formation

A very attractive route to the oxazole is the oxidation of the alcohol prior to ring-closure. The loss of H_2O would then lead directly to the desired oxazole. Test reactions were first performed using compound **63**. Oxidation to the aldehyde **67** was possible with Dess-Martin reagent[216] (*Scheme 5.36*). The conversion was very good according to TLC analysis, and NMR analysis was consistent with the aldehyde product, while in ESI-MS the methanol adduct was observed, demonstrating the high reactivity of **67**. This substance was treated with DAST following the procedure of Ballut[217] and a mixture containing the desired oxazole **121** and the 5-fluorooxazoline **122** was observed due to the absence of base in this reaction.

Scheme 5.36. *Oxidation prior to ring closure: aldehyde or acid*



a) 1.15 equiv. Dess-Martin reagent, CH_2Cl_2, 0°C to rt, N_2, 16 h;
b) 1.25 equiv. DAST, CH_2Cl_2, 0°C to rt, N_2, 1 h.

All other attempts to convert **65** and **64** to the corresponding aldehydes (e.g. **123**, *Scheme 5.36*) failed. Complete oxidation to acid **124** could potentially deliver a precursor for 5-hydroxyoxazole **125**. This is also a very attractive target giving the possibility to install different substituents on the macrocycle. Test reactions in this direction were performed on **64** (*Scheme 5.36*). Whereas $KMnO_4$ did not give complete conversion, a procedure using CrO_3 and HIO_6[218] showed consumption of all starting material **64**. The crude material was treated with Ac_2O and Et_3N[219] but no oxazole product could be isolated. Another oxidation method using TEMPO and phenyliododiacetate (PIDA)[220-222] lead to decomposition. An attempt using CrO_3 and HIO_6 on the macrocycle **59** showed many different products including the starting material. The poor conversion of the alcohol units was also assigned to the poor solubility of **59** in a mixture of H_2O and MeCN. The number of products is probably increased since any acid units will be susceptible to decarbonylation due to the neighbouring π-acceptor (thiazole or ester group) forming a 1,3 dicarbonyl system.

5.3.3.5 Oxazoline ring-closure using TsCl and MsCl

A common method for the synthesis of oxazolines is the activation of the β-alcohol using tosylchloride (or other sulfonylchlorides) followed by intramolecular displacement by the amide group. Dissolving molecules like **63**, **65**, **106**, **104** or **64** in CH_2Cl_2, DMF or pyridine, adding 2 equiv. DMAP or Et_3N and 1 – 1.5 equiv. TsCl gives corresponding oxazolines in yields up to 95%. No tosylated product could be isolated but the formation of elimination side product **71** could sometimes be

observed. With macrocycle **59** this reaction was performed in MeCN using Et_3N and a catalytic amount DMAP. Traces of $[M_{\mathbf{59}} - 4\ H_2O + Na]^+$, $[M_{\mathbf{59}} - 4\ H_2O + TsOH + Na]^+$, $[M_{\mathbf{59}} - 4\ H_2O + 2\ TsOH + Na]^+$ could be observed in ESI-MS among unidentified compounds. All attempts at optimizing this reaction were not successful. DMF, pyridine and DMSO were tested as solvents, and reaction temperatures were screened between -5℃ and 125℃. Variable bases wer e added at the beginning or during the reaction. Mesylchloride was also evaluated, but the results were always the same. The crude material was adsorbed onto SiO_2 and filtered over SiO_2 with solvent mixtures of $CHCl_3$ and MeOH or AcOEt and MeOH but the clean product **61** (*Scheme 5.31*) could not be isolated. It is possible that compound **61** is not very stable and can hydrolyse back on SiO_2 to **59**. The best results were achieved when the crude material from a reaction of macrocycle **59** with MsCl and DMAP at -5℃ in pyridine was filtered over SiO_2, dissolved in DMF and treated again with DMAP to ensure complete formation of tetraoxazoline **61**. The crude material was again filtered over SiO_2 and oxidized using 12 equiv. DBU and 6 equiv. $BrCCl_3$ in DMF. After 24 hours, the solvent was removed and an oily residue was suspended in $CHCl_3$ and filtered. A colourless solid was isolated on the filter and eluted with TFA. This solution contained traces of a substance with a mass consistent with target macrocycle **32**: 601.0 as $[M_{\mathbf{32}} + H]^+$ and 623.0 as $[M_{\mathbf{32}} + Na]^+$ according to MALDI-MS (*Figure 5.13*).

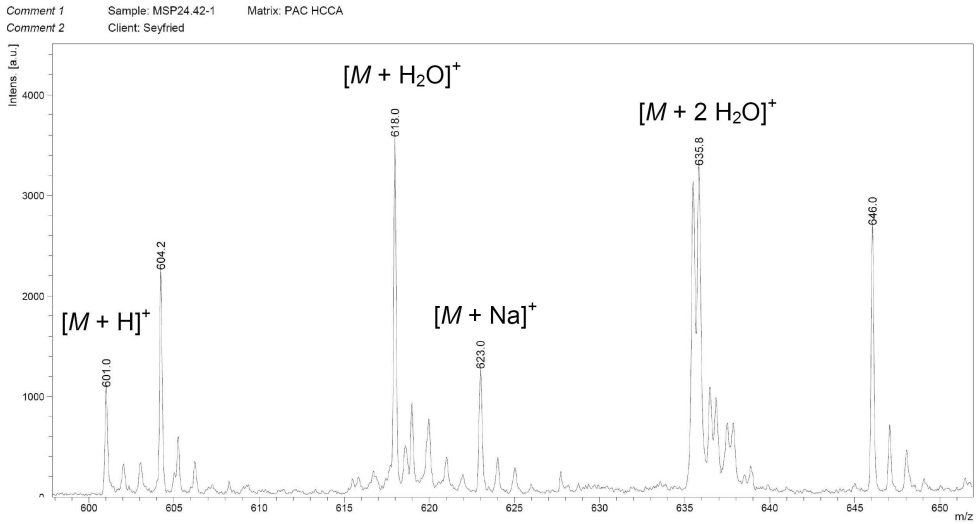
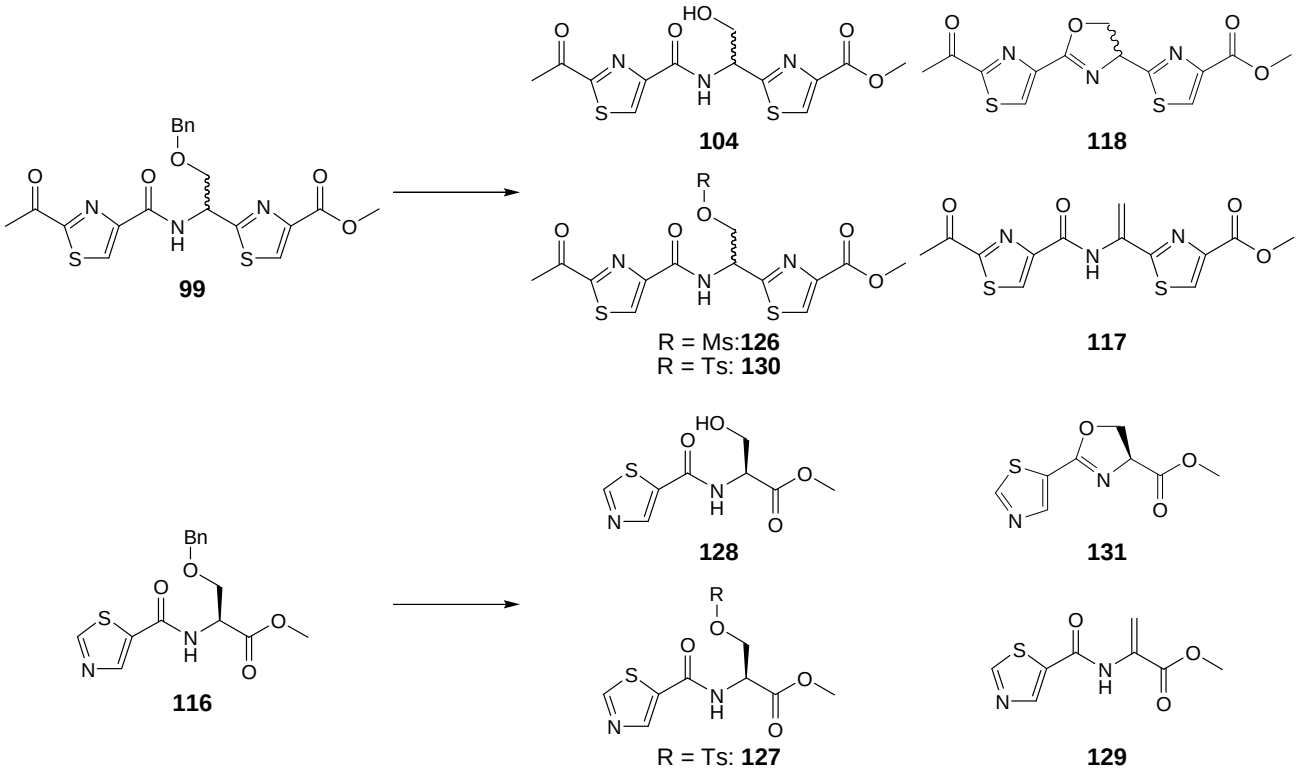


Figure 5.13: *MALDI-MS of a partial conversion to **32** in matrix PAC HCCA*

Further optimization attempts were aimed at alcohol activation using groups that are stable enough to be isolated, yet reactive enough for base-mediated ring-closure

to **61**. **59** was therefore reacted with eight equivalents of mesitylchloride and 12 equiv. Et_3N in MeCN at 60°C. The crude product revealed in ES I-MS signals for $[M_{\mathbf{59}} + Na]^+$, $[M_{\mathbf{59}} - H + Mts + Na]^+$, $[M_{\mathbf{59}} - 2\ H + 2\ Mts + Na]^+$ and $[M_{\mathbf{59}} - 3\ H + 3\ Mts + Na]^+$. There was also a trace of $[M_{\mathbf{61}} + MtsOH + Na]^+$ (831.1) amongst the signals, indicating that ring-closure takes place. Sulfonylation was also attempted using triisopropyl-benzenesulfonylchloride. **59** was reacted with 20 equiv. of the triisopropylbenzenesulfonylchloride and eight equiv. Et_3N in pyridine for 5 h at rt and for 30 min at 85°C. The crude product was isolated upon aqueous workup and fractionated over SiO_2. The desired product could, according to ^{1}H-NMR, not be identified. It is hypothesised that the basic conditions and heat are needed for the formation of the sulfonyl substituted compounds is sufficient for ring-closure and/or elimination reactions to occur.

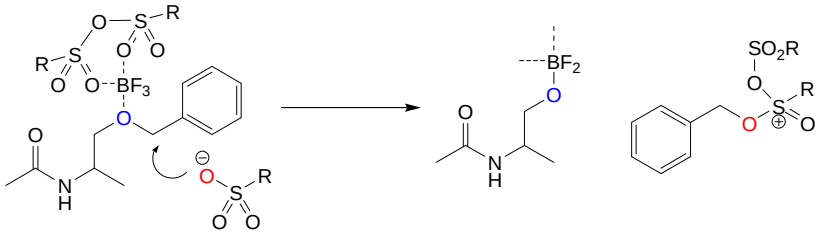
Scheme 5.37. *Debenzylation and sulfonylation of test compounds **99** and **116***



To avoid basic reaction conditions, the benzyl deprotection reaction was modified to generate a stable sulfonyl substituted tetraalcohol in analogy to the formation of the tetraacetate **105** (see chapter 5.2.6). To test this approach, model molecule **99** was reacted with Ms_2O and $BF_3.Et_2O$ in CH_2Cl_2 at rt. The reaction was heated to reflux for 5 h. Following purification, only 15% of the desired, mesylated product **126** could be isolated (*Scheme 5.37*). The main byproduct was oxazoline **118**. Model

compound **116**, Ts_2O and $BF_3.Et_2O$ were reacted in CH_2Cl_2 at rt for 16 h to furnish the desired tosylcompound **127** in approximately 35% isolated yield. As byproducts, alcohol **128** and elimination product **129** were identified (*Scheme 5.37*). The deprotection and tosylation procedure was also tried on **99**. Complete consumption of **99** was observed if the solution was refluxed overnight. A complex mixture including 16% of the tosylated product **130**, and 31% of corresponding oxazoline **131** and alcohol **128** were isolated (*Scheme 5.37*). The whole range of sulfonylation, ring-closure, hydrolysis, and elimination is therefore observed. In the case of macrocycle **58**, Ms_2O was not strong enough to completely remove the benzyl groups, even upon heating in CH_2Cl_2. Considering the probable mechanism of the debenzylation (*Scheme 5.38*) it is hypothesized that a boron-alkoxide complex is the primary product of the deprotection resulting from nucleophilic attack at the benzylic CH_2 group. In a second step the alkoxide reacts with a second anhydride moiety. This presents a conundrum, as the more reactive electrophile (like Ms_2O) will be less nucleophilic towards the boron activated ether. Acetic anhydride appears to provide an optimal balance of reactivity.

Scheme 5.38. *Proposed mechanism of the benzyl deprotection using lewis acid and anhydrides*



One-pot reactions that combine the deprotection of the benzyl group, alcohol activation and ring-closing were developed. Test compound **99** was reacted with Tf_2O, $(CF_3CO)_2O$ or Ms_2O and $BF_3.Et_2O$ in CH_2Cl_2 or $ClCH_2CH_2Cl$. When conversion was detected on TLC, a 1 M solution of DMAP was slowly added to the reaction. When using Ms_2O, the desired oxazoline **118** could be isolated in a yield of about 65%. This approach was attempted using macrocycle **60**. Deprotection worked best using Tf_2O and $BF_3.Et_2O$ in $ClCH_2CH_2Cl$. When complete consumption of **60** was detected using TLC, the solvent was removed under reduced pressure and the remaining BF_3 was removed by a N_2 flow. The crude mixture showed in ESI-MS signals for $[M_{\mathbf{59}} + Na]^+$, $[M_{\mathbf{59}} + H]^+$, $[M_{\mathbf{59}} - H_2O + H]^+$, $[M_{\mathbf{59}} - 2\ H_2O + H]^+$, $[M_{\mathbf{59}} - 3\ H_2O + H]^+$ and $[M_{\mathbf{59}} - 4\ H_2O + H]^+ = [M_{\mathbf{61}} + H]^+$ (*Figure 5.14*). The reaction mixture was

then cooled down to -95℃ and pyridine was added to facilitate ring-closure. According to HPLC this didn't have a significant effect. Tf_2O and DMAP were therefore added[223, 224]. Some material was purified on reversed phase preparative HPLC, and again a mixture of the macrocycle with one or two oxazolines and two or three alcohols was observed. A slight improvement could be achieved by isolating the tetraaclcohol on prep. HPLC, suspending it in CH_2Cl_2 and adding Tf_2O and pyridine at -95℃. Procedures like this increased t he complexity of the mixture. It was unfortunately not possible to get a clean conversion to **61** using this approach.

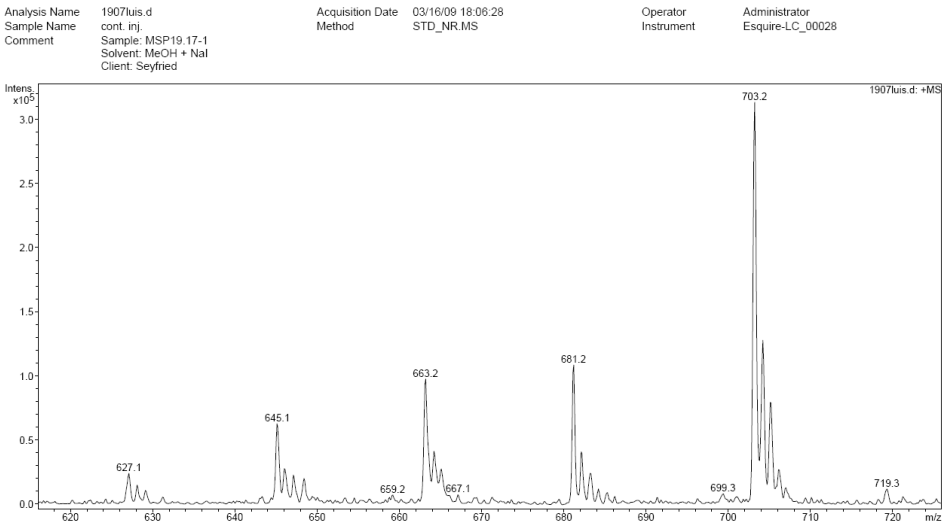
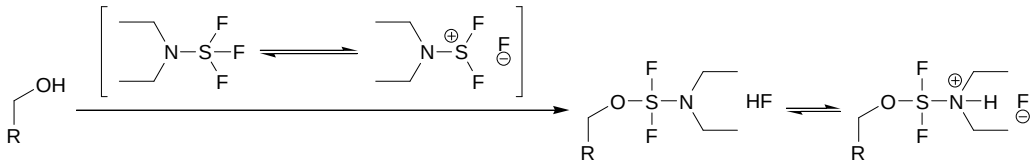


Figure 5.14: *ESI-MS of partial conversion to **61***

5.3.3.6 Ring closure using DAST

The most commonly used reagent for ring-closure of β-hydroxyamides to oxazolines is (diethylamino)sulphurtrifluoride (DAST). While the precise nature of the reactive species of DAST is unknown (*Scheme 5.39*), the alcohol oxygen reacts with the highly electrophilic sulphur. The sulphur substituent on the alcohol activates the oxygen, making the system susceptible to substitution. DAST was originally used for the conversion of alcohols into fluorides as an alternative to gaseous and very toxic SF_4[225-227]. In the context of β-hydroxyamides, the intramolecular attack of the nucleophilic amide oxygen is faster than the substitution by the fluoride. In some examples, the resulting HF is quenched by addition of powdered K_2CO_3. Normally this is not necessary and the produced HF does not react with the products or the equipment, and is removed during aqueous workup[157, 209, 228-230].

Scheme 5.39. *Activation mechanism with the reagent DAST*



In a typical DAST-promoted cyclodehydration reaction, the starting material in dry CH_2Cl_2 is reacted with 1.1 equiv. DAST under dry atmosphere between -20°C and rt. These conditions were successfully applied to the test molecules **63**, **65**, **64**, **104** (*Figure 5.11*) with almost quantitative yields. All these test molecules show good solubility in CH_2Cl_2. Unfortunately, these conditions did not work with tetraalcohol **59** due to its poor solubility in CH_2Cl_2 or $CHCl_3$. Several possible solutions for this problem were considered:

a) Use a different cyclodehydration method compatible with more polar solvents.

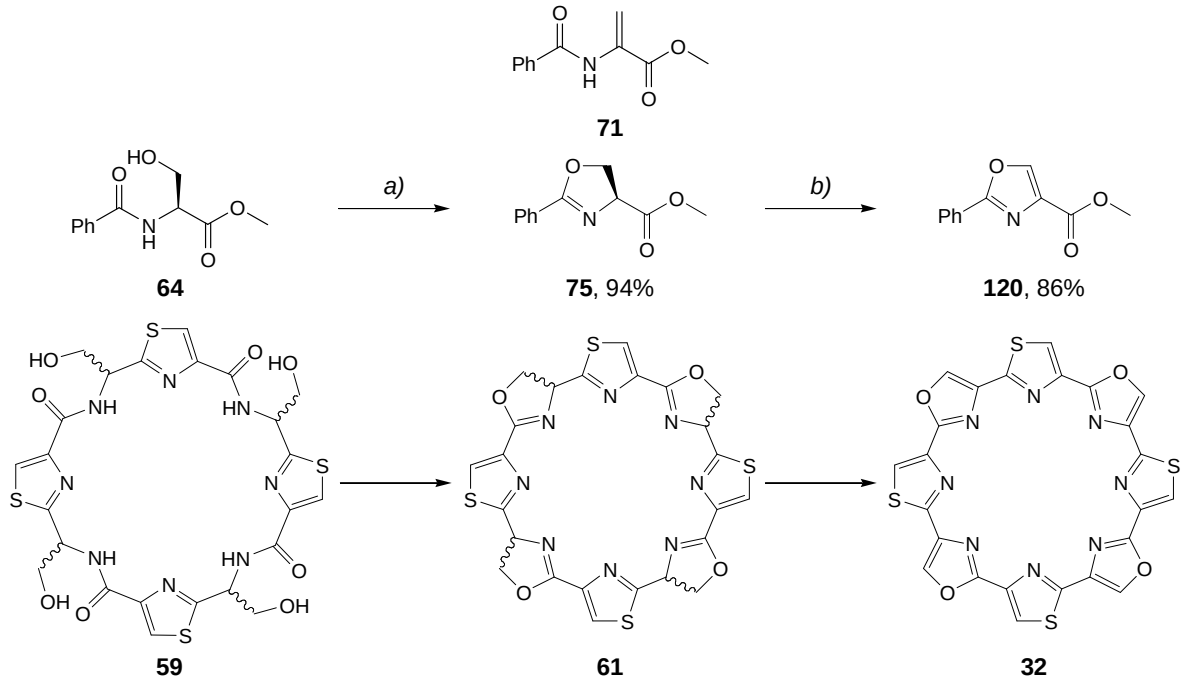
b) Increase the solubility of **59** by addition of chaotropic salts. LiCl in THF was not powerful enough to dissolve **59** but did lead to the discovery of a new chlorination reaction discussed in section 5.3.3.1.

c) Increase the solubility of **59** by protection of the alcohols with silyl protecting groups, which then, upon treatment with DAST, are deprotected due to the presence of fluorides. This will be described in section 5.3.3.7/8.

d) Test the compatibility of DAST with more polar solvents.

A series of DAST-mediated cyclizations was performed with test compound **64**. Using mixture of $CHCl_3$, Et_3N and DAST in a ratio of 10:1:1 worked well, but macrocycle **59** was still insoluble in this solvent mixture. In DMSO, only partial conversion of **64** was observed, but a strong sulphur odour was detected indicating the decomposition of the solvent. In pyridine, ring-closure to give **75** was observed, but formation of the α-β elimination byproduct **71** was also observed. DMF was found to be compatible with DAST, **64** was nicely converted to **75** in 94% yield. Surprisingly, DMAc was much worse than DMF. All these reactions were performed under dry atmosphere (N_2) at -5 to 0°C (*Scheme 5.40*).

Scheme 5.40. *Two-step reaction to the oxazole*



a) 1.2 equiv. DAST, dry DMF, 0°C to rt, N_2, 2 h;
b) 2 equiv. DBU, 1.1 equiv. $BrCCl_3$, DMF, 0°C to rt, N_2, 16 h.

Macrocycle **59** is highly soluble in DMF and was consumed upon addition of DAST. Isolation of the resulting products proved to be difficult. Reversed phase HPLC using a H_2O/MeCN gradient showed a mixture of many different products. On TLC, traces of less polar products were detected. These products appeared to be incompatible with aqueous workup, and were not detected on TLC anymore after workup. A subsequent oxidation step was therefore attempted immediately after the cyclization step without purification. The two-step, one-pot reaction was first evaluated with test substance **64**. The reaction was monitored on TLC and showed a clean conversion to oxazole **120**. Addition of powdered K_2CO_3 to the reaction promoted β-elimination to give **71** as a byproduct. The procedure was repeated with macrocycle **59** that was dissolved in DMF to a concentration of 15 mM and cooled down to -5°C in an ice-brine bath. Eight equivalents DAST were added, and the solution was stirred under N_2 and allowed to slowly warm to rt. After 4 h the solution was cooled down to -5°C, and 20 equiv. of DBU and eight equiv. of $BrCCl_3$ were added. Three hours later the mixture was worked up under aqueous conditions. MALDI-MS analysis of the crude product includes signals belonging to the desired molecule and a ladder of signals 601.1, 603.1, 605.1, 607.1, 609.1 belonging to $[M_{\mathbf{32}} + H, + 3\ H, + 5\ H, + 7\ H, + 9\ H]^+$, the last mass is the same as $[M_{\mathbf{61}} + H]^+$. The second and third ladders at higher masses belong to compounds that were not fully cyclodehydrated: 619.1, 621.1,

623.0, 625.0 belongs to [{M_{32} + H_2O} + H, + 3 H, + 5 H]$^+$ and 637.1, 639.1 to [{M_{32} + 2 H_2O} + H, + 3 H]$^+$ (*Figure 5.15*). Given the complex mixture of products, this strategy was not pursued further.

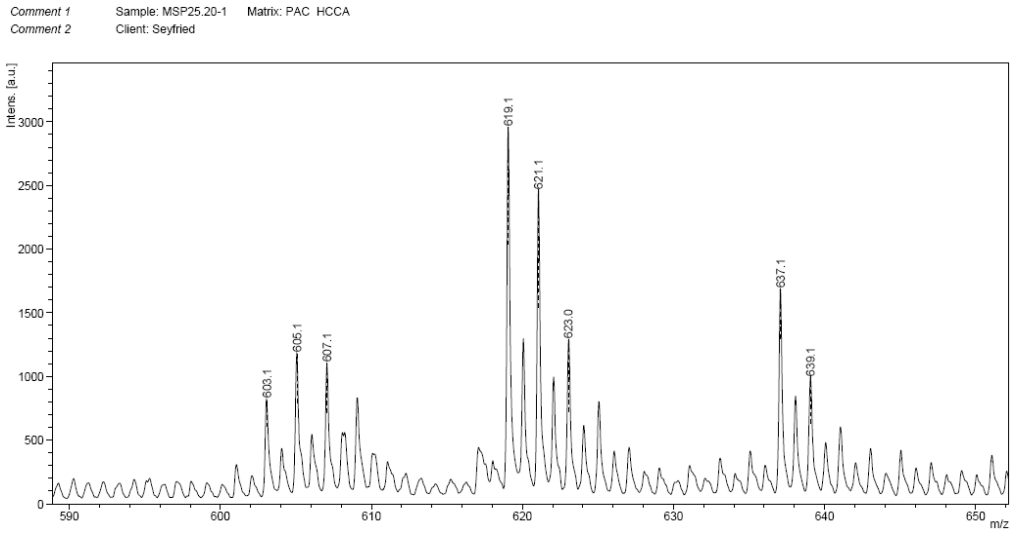


Figure 5.14: *MALDI-MS of a partial reaction to **32***

5.3.3.7 Silyl protected alcohols as oxazoline precursors

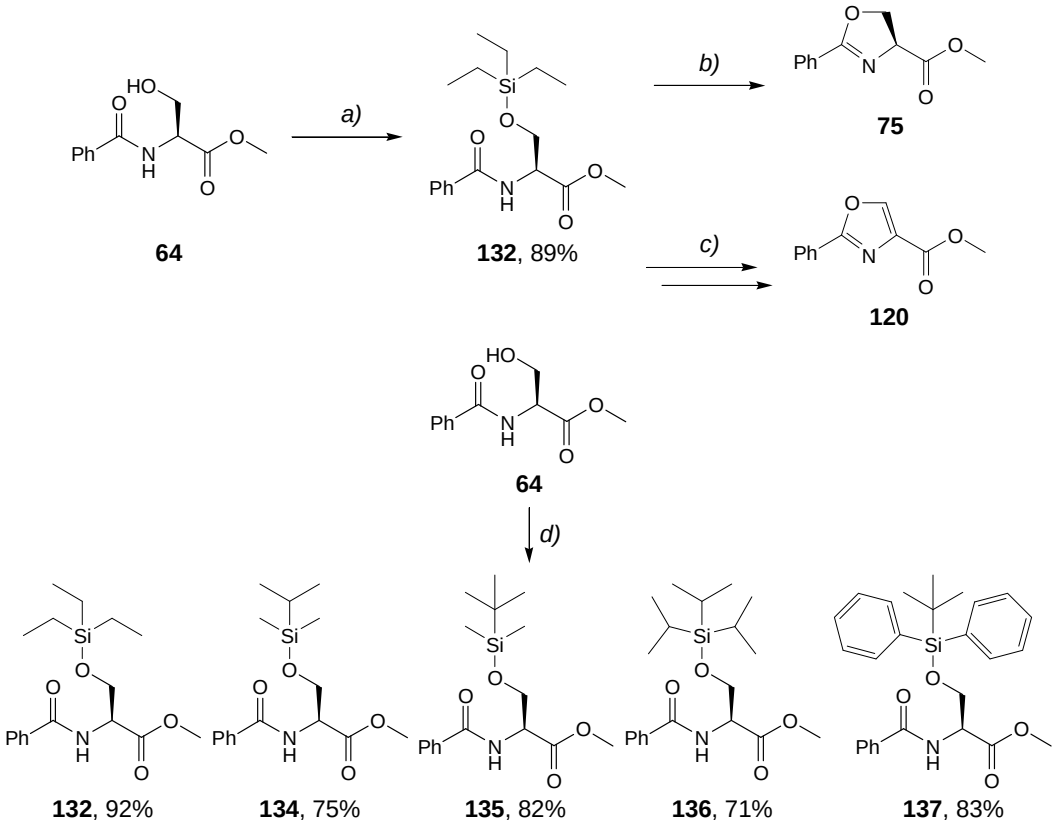
In the previously described cyclodehydration reactions we have hypothesized that the failure for complete conversion from tetraalcohol **59** to tetraoxazoline **61** is related to poor solubility properties of macrocycle **59**. We were therefore interested in a new concept of using silyl protected β-hydroxyamides for DAST mediated cyclodehydration reactions[231, 232]. According to this approach, silyl groups are used to protect alcohol groups on the macrocycle to help increase its solubility in organic solvents. Upon addition of DAST, the alcohols are then slowly deprotected due to the fluoride ions that result from cyclodehydration. The deprotected alcohols then react quickly with DAST to form the oxazolines. The rate of deprotection should be controllable using different silyl groups. To keep the macrocycle in solution, the rate of deprotection should be slower than the rate of ring-closure.

Preliminary experiments were performed with test compound **64**. It was reacted with 5 equiv. chlorotriethylsilane in pyridine at 60°C to give the TES protected compound **132** in a yield of around 88% (*Scheme 5.41*). **132** was reacted with 1.1 equiv. DAST to quantitatively furnish oxazoline **75**. When completion was reached according to TLC, 3 equiv. DBU and 1 equiv. $BrCCl_3$ were added to oxidize this oxazoline to the oxazole **120** (*Scheme 5.41*). Doing the same reaction in $CHCl_3$, the

conversion was less clean, and in CH_2Cl_2 containing K_2CO_3 the elimination side product **71** was observed.

Macrocycle **59** was protected with TES groups using 20 equiv. TESCl in pyridine at 60° for 2 h. Compound **133** could be isolated in yields around 50% (*Scheme 5.42*). Macrocycle **133** was soluble in CH_2Cl_2 and was treated with 20 equiv. DAST in CH_2Cl_2 at 0°C. After 5 min, the formation of a precipitate was observed. This effect was ascribed to deprotection of several TES groups that occurs much faster than the reaction with DAST and formation of the oxazoline. As a consequence, a complex mixture of products was obtained. An alternative cyclodehydration reagent was therefore needed.

Scheme 5.41. *Protection with silyl groups to do the ring-closure to the oxazole*

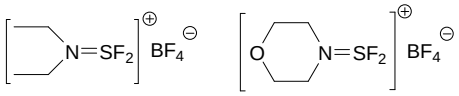


a) 5 equiv. TESCl, pyridine, 60°C, N_2, 1 h; b) 1 equiv. DAST, CH_2Cl_2, rt, N_2, 20 min; c) 1.1 equiv. DAST, CH_2Cl_2, rt, N_2, 20 min, 2. 3 equiv. DBU, 1 equiv. $BrCCl_3$, CH_2Cl_2, 0°C to rt, N_2, 30 min.; d) 1.2 equiv. silylchloride, 2 equiv. imidazole, DMF, rt, N_2, 16 h.

5.3.3.8 Silanols and XtalFluor for the synthesis of oxazolines

XtalFluor compounds (*Figure 5.16*) are next-generation fluorinating agents recently described by Michel Couturier[233]. They are more easily handled and significantly more stable than DAST. They should be more selective and exhibit superior

performance since they show less tendency to form the elimination side products[234]. The combination of silyl protected alcohols and XtalFluors for cyclodehydration reactions is therefore very attractive since it allows the use of less polar solvents together with a slow *in situ* deprotection of the alcohols and fast conversion to the oxazoline. To succeed, the rate of deprotection should be slower than the rate of the cyclization reaction. Otherwise, the intermediate alcohols or alkoxides might precipitate. The silyl group should therefore be chosen carefully. Different silyl groups were screened in reactions with DAST and XtalFluor. The following work was performed by Fabian Roth during his master thesis in our lab.



XtalFluor-E XtalFluor-M

Figure 5.16: *Crystalline fluorinating agents XtalFluor-E and -M*

Model compound **64** was protected using triethylsilylchloride (TESCl), isopropyldimethylsilylchloride (IPDMSCl), *tert*-butyldimethylsilylchloride (TBDMSCl), triisoproylsilylchloride (TIPSCl) and *tert*-butyldiphenylsilylchloride (TBDPSCl) with 2 equiv. imidazole in DMF at rt overnight to furnish the respective compounds **132**, **134**, **135**, **136**, **137** in yields between 70 and 90%[235] (*Scheme 5.41*).

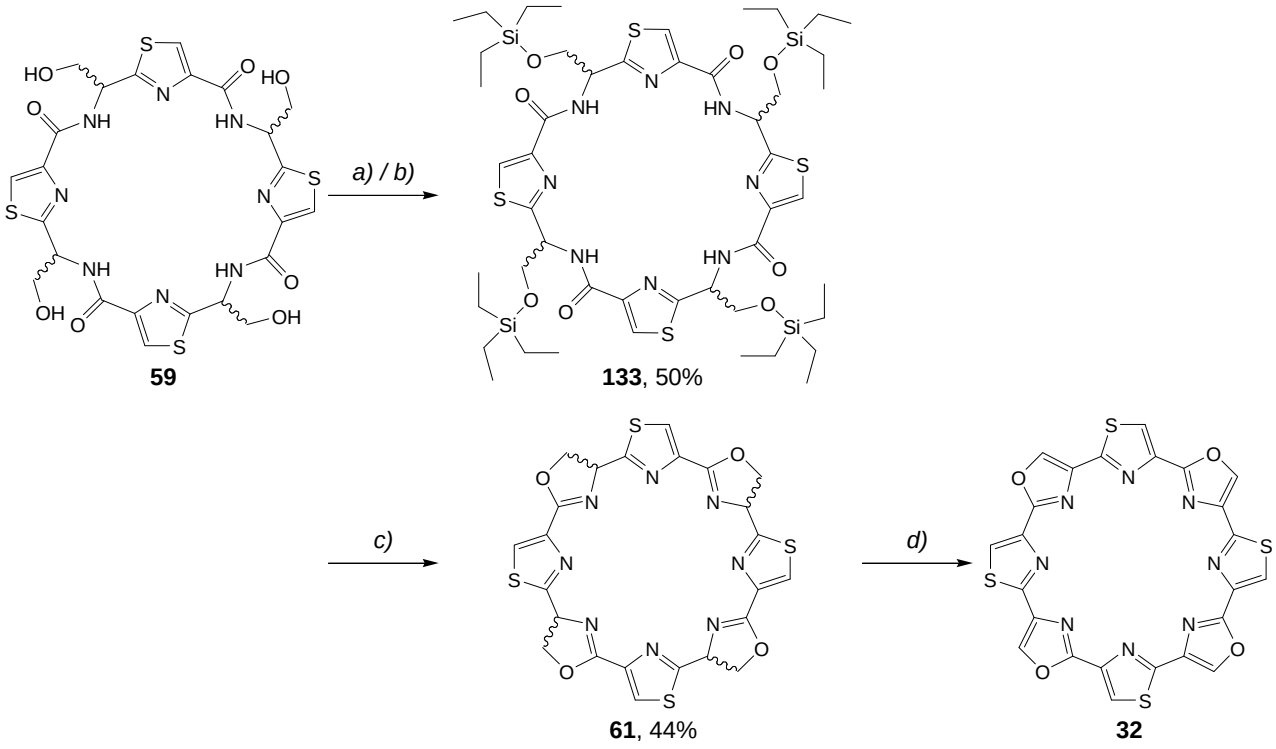
Using these compounds following trends for DAST or XtalFluor mediated oxazoline formation were observed:

- For both DAST and XtalFluors, the intermediate alcohol could not be observed on TLC;
- The rate of deprotection and ring-closure was fastest for TES and became slower for more sterically-hindered silyl groups;
- For all silyl groups that are less reactive than IPDMS, reactions did not reach completion within 72 h.
- XtalFluors exhibit a longer half-life in solution than DAST and are therefore more compatible with slower reactions.

The XtalFluor-mediated oxazoline formation was performed using the TES protected macrocycle **133**. Reactions in CH_2Cl_2 (15 mM) using 16 equiv. of XtalFluor-E which was added in one portion resulted according to TLC in clean conversion within 16 h. The solvent was removed, and the remaining material was adsorbed onto oven-dried neutral AlO_3 and chromatographically purified on neutral AlO_3. About

44% of the macrocycle **61** could be isolated as a mixture of diastereoisomers (*Scheme 5.42*). Oxidation to the target molecule **32** was performed by adding 12 equiv. $BrCCl_3$ to a 3 mM solution of **61** in DMF and adding then 36 equiv. of DBU as a 50 mM solution in DMF over 10 min at 0℃. Immedia tly after the addition of $BrCCl_3$ the formation of a precipitate was observed, but the reaction was allowed to proceed overnight at rt. The product was isolated by filtration washing the solid with $CHCl_3$, AcOEt and MeOH. **32** is slightly soluble in DMSO and highly soluble in TFA. In MALDI-MS the compound was identified as 601.0 $[M_{\mathbf{32}} + H]^+$ and 622.9 $[M_{\mathbf{32}} + Na]^+$ (*Figure 5.17*). ^{1}H-NMR shows only two signals, consistently with a planar structure of **32** (*Figure 5.18*).

Scheme 5.42. *Series of silyl protected β-hydroxyamides for XtalFluor-mediated ring-closures*



59 **133**, 50% **61**, 44% **32**

a) 16 equiv. TESCl, pyridine, 60℃, N_2, 2 h; b) 4.8 equiv. TESCl, 8 equiv. imidazole, DMF, rt, N_2, 16 h; c) 16 equiv. XtalFluor-E, CH_2Cl_2, rt, N_2, 16 h; d) 12 equiv. $BrCCl_3$, 36 equiv. DBU, DMF, 0℃ to rt, N_2, 16 h.

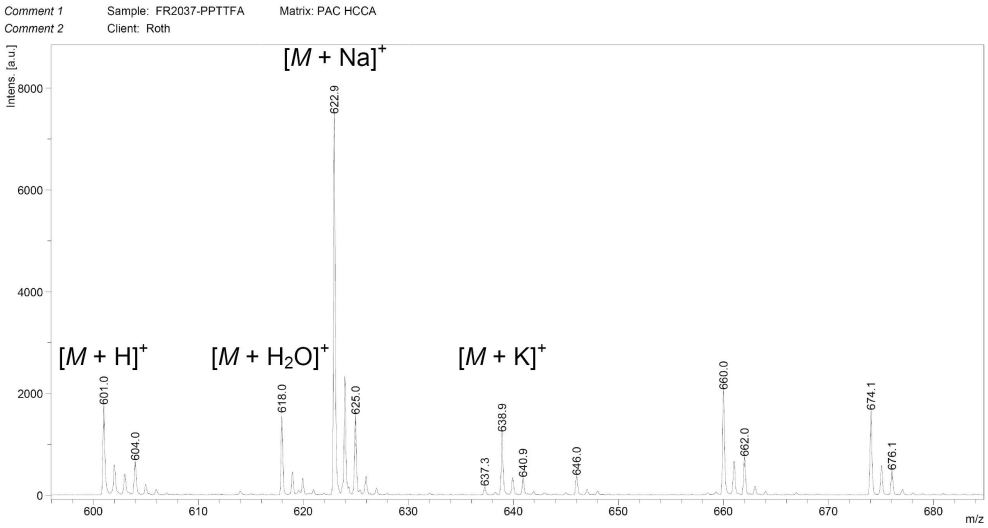


Figure 5.17: *MALDI-MS of 4TOP (**32**) in matrix PAC HCCA*

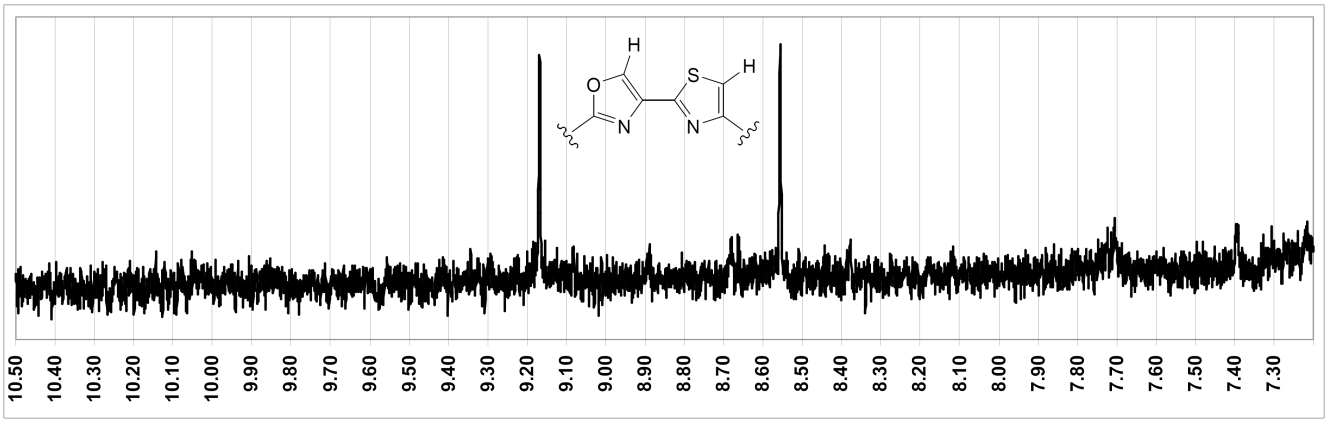


Figure 5.18: *^{1}H-NMR of 4TOP (**32**) in d_6-DMSO*

6. Outlook

Product **32** exhibits partial solubility in DMSO giving the possibility for biological evaluation. To characterize macrocycle **32**, our lab is currently testing:

a) Crystallization with and without G-quadruplexes;
b) G-quadruplex affinity by fluorescence quenching assay;
c) G-quadruplex specificity by comparing titrations using different DNA sequences known to form G-quadruplexes or duplex DNA;
d) Cytotoxicity in cells by growing experiments in the presence of macrocycle **32**;
e) Impact on global gene expression by gene chip arrays.

The results from these experiments will evaluate the potential applications of planar telomestatin analogs in medicine and bioanalytics. Good characteristics in terms of affinity to and specificity for G-quadruplexes will motivate diversification of this scaffold. Substitution of the macrocycle, for example in the 5-position of four azole units, can potentially influence the binding preferences towards different G-quadruplexes and the pharmacokinetic and toxicological properties of these compounds.

8 Synthetic Procedures and Analytical Data

8.0 Synthesis of model compounds

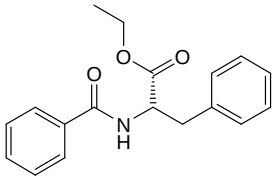
8.0.1 Models for β-hydroxyamides

8.0.1.1 Synthesis of **63**

8.0.1.1.1 Synthesis of **138**

3.01 g of L-phenylalanine ethyl ester hydrochloride (13.1 mmol) were suspended in 60 ml THF in a dry two necked flask with a thermometer. 9.0 ml Et_3N (65 mmol, 5 equiv.) were added and 1.7 ml benzoylchloride (15 mmol, 1.1 equiv.), dissolved in 60 ml THF, were dropped in slowly with a dropping funnel. The temperature was kept at 25℃. The reaction was monitored on TLC (H:AcOEt 5: 1). To complete the conversion, the solution was heated up to reflux for 1 h. The solution was then diluted with AcOEt, washed two times with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, the comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv and hv. 4.13 g (12.5 mmol, 96%) of the crude product were isolated. 3.62 g (11.0 g, 84%) of the pure *N*-benzoyl-L-phenylalanine ethyl ester (**138**) were obtained after recrystallisation from H:AcOEt 10:1.

R_f(H/AcOEt 5:1): 0.32



138

^{1}H-NMR (300 MHz, $CDCl_3$): 7.74 – 7.13 (*m*, 10 H, arom. CH); 6.61 (br. *d*, 3*J* = 7.2, 1 H, NH); 5.07 (*dt*, ^3J = 7.5, ^3J = 5.6, 1 H, NCH); 4.21 (*q*, ^3J = 7.1, 2 H, OCH_2); 3.30 (*dd*, ^2J = 13.8, ^3J = 5.8, 1 H, CH_2Ph); 3.23 (*dd*, ^2J = 13.8, ^3J = 5.4, 1 H, CH_2Ph); 1.28 (*t*, ^3J = 7.1, 3 H, CH_3).

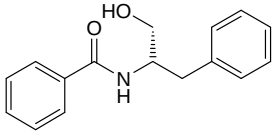
^{13}C-NMR (75.5 MHz, $CDCl_3$): 171.62 (*s*, 1 C, COOEt); 166.79 (*s*, 1 C, CONH); 135.91; 133.98 (2 *s*, 2 C, 2 arom. C); 131.75; 129.43; 128.62; 128.56; 127.15; 127.00 (6 *d*, 10 C, arom. CH); 61.65 (*t*, 1 C, OCH_2); 53.54 (*d*, 1 C, NCH); 37.95 (*t*, 1 C, CH_2Ph); 14.15 (*q*, 1 C, CH_3).

MS ($CHCl_3$ / MeOH 4:6 + NaI): 320.1 (100, $[M + Na]^+$).

8.0.1.1.2 Synthesis of **63**

3.01 g of **138** (9.14 mmol) were dissolved in 100 ml THF (solv. syst.) under N_2 and cooled down to 0℃ in an ice bath. 6.8 ml 2 M $LiBH_4$ solution in THF (13.6 mmol, 1.5 equiv.) were added and the solution was slowly let warm up to rt while stirring. The change could be monitored on TLC (H:AcOEt 1:1). The remaining $LiBH_4$ was quenched by adding drops of H_2O and 0.1 M citric acid. A part of the THF was then removed by rv and a precipitate formed. The aq. suspension was extracted once with AcOEt and once with TBME. The comb. org. layers were washed once with sat. $NaHCO_3$ and once with brine, dried over $MgSO_4$, filtered and solvent removed in rv and hv. The product was purified by recrystallisation from toluene. 1.81 g of the pure *N*-(1-hydroxy-3-phenylpropan-2-yl)benzamide (**63**) (7.09 mmol, 93%) were isolated.

Melting temperature: 169.2 – 170.7°C
R_f(H/AcOEt 1:1): 0.30


63

^{1}H-NMR (300 MHz, $CDCl_3$ / 10% MeOD): 7.74 – 7.18 (*m*, 10 H, arom. CH); 4.34 (*ddd*, ^3J = 11.8, ^3J = 7.3, ^3J = 4.5, 1 H, NCH); 3.96 (br. *s*, 2 H, OH & NH); 3.69 (*dd*, ^2J = 11.5, ^3J = 4.2, 1 H, OCH_2); 3.63 (*dd*, ^2J = 11.5, ^3J = 5.0, 1 H, OCH_2); 3.01 (*dd*, ^2J = 13.8, ^3J = 7.3, 1 H, CH_2Ph); 2.95 (*dd*, ^2J = 13.8, ^3J = 6.5, 1 H, CH_2Ph).

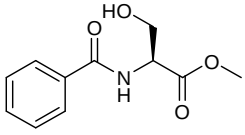
^{13}C-NMR (75.5 MHz, $CDCl_3$ / 10% MeOD): 168.29 (*s*, 1 C, CONH); 137.80; 134.09 (2 *s*, 2 C, arom. C); 131.32; 129.03; 128.23; 126.74; 126.24 (5 *d*, 10 C, arom. CH); 62.65 (*t*, 1 C, OCH_2); 52.84 (*d*, 1 C, NCH); 36.62 (*t*, 1 C, CH_2Ph).

MS (ESI, MeOH): 278.1 (7, $[M + Na]^+$); 256.1 (100, $[M + H]^+$).

8.0.1.2 Synthesis of **64**

2.01 g of H-Ser-OMe.HCl (12.9 mmol) were suspended in 75 ml CH_2Cl_2 (*puriss. p.a.*) and cooled down to 0°C in an ice bath under N_2. 1.5 ml benzoylchloride (13 mmol, 1 equiv.) were added. 3.6 ml Et_3N (26 mmol, 2 equiv.) were mixed with 40 ml CH_2Cl_2 (*puriss. p.a.*) and added over a time of 10 min to the suspension using a dropping funnel[229]. After stirring the suspension for 20 h at rt solvent was removed in rv, the remaining oily residue diluted with AcOEt, washed once with 0.1 M aq. citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, the org. layers combined and dried over $MgSO_4$. Solid was filtered off and solvent removed in rv. The crude solid **64** was purified by recrystallisation from ~30 ml refluxing mixture of hexane/CH_2Cl_2 1:1. 2.14 g of the pure colourless crystalline product methyl (2*S*)-2-(benzoylamino)-3-hydroxypropanoate (**64**) (9.60 mmol, 74%) were isolated.

Melting temperature: 87.1 – 89.2°C
R_f(H/AcOEt 1:1): 0.07, R_f(H/AcOEt 1:3): 0.25
$[\alpha]_D^{20}$ ($CHCl_3$): + 46.7


64

^{1}H-NMR (300 MHz, $CDCl_3$): 7.84 – 7.81 (*m*, 2 H, arom. *o*-CH); 7.55 – 7.50 (*m*, 1 H, arom. *p*-CH); 7.46 – 7.41 (*m*, 2 H, arom. *m*-CH); 7.18 (br. *d*, ^3J = 6.7, 1 H, NH); 4.86 (*dt*, ^3J = 7.2, ^3J = 3.6, 1 H, NCH); 4.08 (*dd*, ^2J = 11.3, ^3J = 3.7, 1 H, OCH_2); 4.03 (*dd*, ^2J = 11.3, ^3J = 3.5, 1 H, OCH_2); 3.81 (*s*, 3 H, OCH_3); 2.92 (br. *s*, 1 H, OH).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 171.04 (*s*, 1 C, COOMe); 167.67 (*s*, 1 C, CONH); 133.40 (*s*, 1 C, arom. C); 131.98 (*d*, 1 C, arom. *p*-CH); 128.61 (*d*, 2 C, arom. *o*-CH); 127.15 (*d*, 2 C, arom. *m*-CH); 63.43 (*t*, 1 C, CH_2OH); 55.13 (*d*, 1 C, NCH); 52.85 (*q*, 1 C, OCH_3).

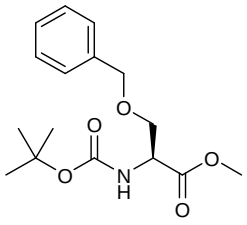
MS (ESI-MS, MeOH + NaI): 246.1 (100, $[M + Na]^+$).

8.0.1.3 Synthesis of **116**

8.0.1.3.1 Synthesis of **139**

2.00 g of Boc-Ser(OBn)-OH (6.72 mmol), 1.54 g of DCC (7.47 mmol, 1.1 equiv.) and 83 mg of DMAP (0.68 mmol, 0.1 equiv.) were suspended in 45 ml dry MeOH (*puriss. p.a.*). The mixture was stirred at rt under N_2 overnight[189]. The progress of the reaction was monitored on TLC (H:AcOEt 3:1). The solution was then filtered over Celite, the solvent removed in rv and the remaining material dissolved in AcOEt, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and the solvent removed in rv. The crude product was purified on SiO_2 (H:AcOEt 4:1). 1.99 g of the pure product Boc-Ser(OBn)-OMe (**139**) (6.44 mmol, 95%) were isolated as colourless crystals.

R_f(H/AcOEt 3:1): 0.5



139

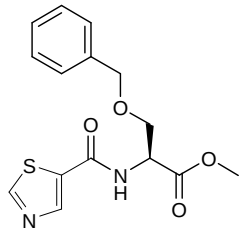
^{1}H-NMR (300 MHz, $CDCl_3$): 7.37 – 7.26 (*m*, 5 H, arom. CH); 5.40 (br. *d*, ^3J = 8.5, 1 H, NH); 4.55; 4.48 (2 *d*, ^2J = 12.3, 2 H, OCH_2Ph); 4.47 – 4.42 (*m*, 1 H, NCH); 3.87 (*dd*, ^2J = 9.4, ^3J = 3.1, 1 H, OCH_2C); 3.74 (*s*, 3 H, OCH_3); 3.68 (*dd*, ^2J = 9.4, ^3J = 3.2, 1 H, OCH_2C); 1.45 (*s*, 9 H, $OC(CH_3)_3$).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 171.13 (*s*, 1 C, COOMe); 155.44 (*s*, 1 C, Carbamate-CO); 137.51 (*s*, 1 C, arom. C); 128.37; 127.77; 127.54 (3 *d*, 5 C, arom. CH); 79.92 (*s*, 1 C, $OCMe_3$); 73.17 (*t*, 1 C, OCH_2Ph); 69.90 (*t*, 1 C, OCH_2); 53.94 (*d*, 1 C, NCH); 52.38 (*q*, 1 C, OCH_3); 28.26 (*q*, 3 C, $OC(CH_3)_3$).

8.0.1.3.2 Synthesis of **116**

669 mg of **139** (2.16 mmol) were dissolved in 6 ml CH_2Cl_2 and 2 ml TFA were added. The solution was stirred under N_2 for 3 h, then TLC (H:AcOEt 3:1) showed complete conversion. The solvent was removed under reduced pressure freezing it out in a cooling trap. The remaining material was dissolved in MeOH, some of the ion exchanger Dowex in its Cl^--form was added and the mixture stirred overnight. The solid was then removed by filtration and the solvent in rv. The free amine was directly used for peptide bond formation.
279 mg of 1,2-thiazole-4-carboxylic acid (2.16 mmol, 1 equiv.) were suspended in 10 ml CH_2Cl_2 (*puriss*. *p.a.*) and 0.6 ml NMM (5.4 mmol, 2.5 equiv.) were added.
2.16 mmol of the amine were dissolved in 10 ml CH_2Cl_2 (*puriss*. *p.a.*), 0.6 ml NMM (5.4 mmol, 2.5 equiv.), 694 mg of TBTU (2.16 mmol, 1 equiv.) and the solution of the 1,2-thiaole-4-carboxylic acid were added. The solution was stirred at rt under N_2 overnight. The progress of the conversion was monitored on TLC (H:AcOEt 1:1).
The solvent was removed in rv, the remaining material dissolved in AcOEt, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, the comb. org. layers dried over $MgSO_4$, filtered and the solvent removed in rv. The crude material was chromatographically purified on SiO_2 (H:AcOEt 3:1 to 1:1). 660 mg of the pure product methyl 3-(benzyloxy)-2-[(1,3-thiazol-5-yl)carbonyl]propanoic acid (**116**) (2.06 mmol, 95%) were isolated as colourless crystals.

R_f(H/AcOEt 1:1): 0.45, R_f(H/AcOEt 3:1): 0.15
$[\alpha]_D^{20}$ ($CHCl_3$): + 11.9


116

^{1}H-NMR (300 MHz, $CDCl_3$): 8.80 (*d*, ^4J = 1.9, 1 H, Thiazole-C^{2}H); 8.20 (*d*, ^4J = 1.9, 1 H, Thiazole-C^{4}H); 8.17 (br. *d*, ^3J = 8.7, 1 H, NH); 7.34 – 7.28 (*m*, 5 H, arom. CH); 4.96 (*dt*, ^3J = 8.8, ^3J = 3.4, 1 H, NCH); 4.61; 4.54 (2 *d*, ^2J = 12.3, 2 H OCH_2Ph); 4.01 (*dd*, ^2J = 9.5, ^3J = 3.6, 1 H, OCH_2); 3.81 (*dd*, ^2J = 9.5, ^3J = 3.5, 1 H, OCH_2); 3.79 (*s*, 3 H, OCH_3).

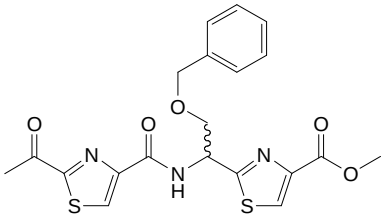
^{13}C-NMR (75.5 MHz, $CDCl_3$): 170.44 (*s*, 1 C, COOMe); 160.67 (*s*, 1 C, CONH); 152.81 (*d*, 1 C, Thiazole-C^{2}H); 150.54 (*s*, 1 C, Thiazole-C^5); 137.55 (*s*, 1 C, arom. C); 128.41; 127.80; 127.62 (3 *d*, 5 C, arom. CH); 123.74 (*d*, 1 C, Thiazole-C^{4}H); 73.24 (*t*, 1 C, OCH_2Ph); 69.60 (*t*, 1 C, OCH_2); 57.02 (*d*, 1 C, NCH); 52.63 (*q*, 1 C, OCH_3).

MS (ESI, MeOH + NaI): 375.1 (6); 360.4 (13); 343.1 (100, [*M* + Na]$^+$).

8.0.1.4 Model compound **99**

Methyl 2-{1-[(2-Acetyl-1,3-thiazole-4-yl)carbonylamino]-2-(benzyloxy)ethyl}-1,3-thiazole-4-carboxylate (**99**) was isolated as a side product.

R_f(H/AcOEt 1:1): 0.2; R_f(H/AcOEt 1:3): 0.65
IR (KBr): 3394*s*; 3120*w*; 3100*m*; 3066*w*; 3033*w*; 2958*w*; 2924*w*; 2872*w*; 1713*s*; 1681*s*; 1524*s*; 1499*s*; 1473*s*; 1455*m*; 1426*m*; 1384*m*; 1366*m*; 1355*m*; 1327*s*; 1305*m*; 1265*m*; 1243*s*; 1208*m*; 1177*m*; 1140*m*; 1081*s*; 1054*m*; 1009*m*; 980*m*; 958*w*; 940*m*; 913*w*; 894*w*; 883*w*; 861*m*; 839*w*; 818*w*; 802*w*; 785*m*; 765*m*; 753*s*; 723*w*; 699*m*; 671*w*; 647*w*; 632*m*; 594*m*; 579*m*; 560*w*; 549*w*; 525*w*; 505*w*; 494*w*; 474*w*; 461*w*.


99

^{1}H-NMR (300 MHz, $CDCl_3$): 8.45 (*s*, 1 H, Thiazole-CH); 8.31 (br. *d*, 1 H, NH); 8.15 (*s*, 1 H, Thiazole-CH); 7.30 – 7.26 (*m*, 5 H, arom. CH); 5.72 (*dt*, ^3J = 8.2, ^3J = 4.7, 1 H, NCH); 4.60 (*s*, 2 H, OCH_2Ph); 4.21 (*dd*, ^2J = 9.4, ^3J = 4.2, 1 H, OCH_2); 3.98 (*dd*, ^2J = 9.4, ^3J = 5.0, 1 H, OCH_2); 3.96 (*s*, 3 H, OCH_3); 2.76 (*s*, 3 H, $(CO)CH_3$).

MS ($CHCl_3$ / MeOH 4:6 + NaI): 468.1 (100, [*M* + Na]$^+$).

8.0.2 Models for β-tosylaminoamide

8.0.2.1 Synthesis of **66**

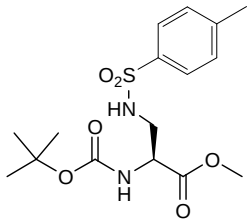
8.0.2.1.1 Synthesis of **140**

2.94 g of **49**[1] (5.84 mmol) were dissolved in 50 ml Et_2O under N_2 and cooled down to 0℃. 24 ml of the ~0.25 M CH_2N_2-solution were added (~6 mmol, 1.1 equiv.). An intense evolution of gas (N_2) was observed and the yellow colour of the diazomethane was instantly lost. Another 2 ml of the ~0.25 M CH_2N_2-solution were

[1] The synthesis of **49** is described in section 8.1.2.3

added. The colour remained partially. The solution was stirred for 10 min and 50 ml of 0.1 M aq. citric acid was added to quench the remaining diazomethane. The layers were separated, the org. one was washed again with 50 ml of 0.1 M aq. citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with Et_2O, the comb. org. layers dried over $MgSO_4$, filtered and dried in rv and hv. The crude product was chromatographically purified on SiO_2 (H:AcOEt 2:1). 1.74 g (4.68 mmol, 80%) of the pure methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(4-toluenesulfonylamino)propionate (Boc-DAP(NTs)-OMe, **140**) were isolated.
The structure of **140** was confirmed by x-ray crystallography.

R_f(H/AcOEt 2:1): 0.4



140

^{1}H-NMR (300 MHz, $CDCl_3$): 7.73; 7.31 (2 *d*, ^3J = 8.4, 4 arom. H); 5.45 (br. *d*, ^3J = 7.3, 1 H, Amide-NH); 5.31 (br. *t*, ^3J = 6.5, 1 H, Ts-NH); 4.35 (br. *s*, 1 H, NCH), 3.73 (*s*, 3 H, OMe); 3.41 – 3.27 (*m*, 2 H, NCH_2); 2.42 (*s*, 3 H, Ts-Me); 1.43 (*s*, 9 H, $C(CH_3)_3$).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 170.48 (*s*, 1 C, COOMe); 155.31 (*s*, 1 C, Carbamat-CO); 143.50 (*s*, 1 C, Ts-C-Me); 136.71 (*s*, 1 C, Ts-C-S); 129.68; 126.95 (2 *d*, 4 C, arom. CH); 80.38 (*s*, 1 C, $OC(CH_3)_3$); 53.39 (*d*, 1 C, NCH); 52.70 (*q*, 1 C, OCH_3); 44.68 (*t*, 1 C, NCH_2); 28.13 (*q*, 3 C, $C(CH_3)_3$); 21.38 (*q*, 1 C, Ts-CH_3).

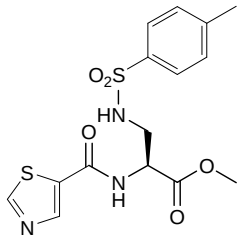
X-ray crystallography: A nice crystal of **140**, suitable for x-ray analysis, was obtained by evaporation of $CDCl_3$ from an NMR tube.

8.0.2.1.2 Synthesis of **66**

1.08 g of **140** (2.90 mmol) were dissolved in 10 ml dry CH_2Cl_2 (freshly distilled over CaH_2). A mixture of 5 ml CH_2Cl_2 and 5 ml TFA were added and the solution was stirred at rt for 3 h under N_2, till TLC (H:AcOEt 1:1, staining with ninhydrin), showed no more **140**. Solvent was removed by rv freezing it out in a cooling trap. The oily residue was dissolved in MeOH, mixed with Amberlyst-A26 in its Cl^--form for 5 h, filtered and solvent removed again in rv. The isolated free amine was directly used for peptide bond formation.

374 mg of 1,3-thiazol-5-carboxylic acid (2.90 mmol) were dissolved in 20 ml CH_2Cl_2 (*puriss*. *p.a.*). 0.81 ml of NMM (7.2 mmol, 2.5 equiv.), 931 mg of TBTU (2.90 mmol, 1 equiv.) and 2.90 mmol of the amine (1 equiv.), dissolved in 20 ml CH_2Cl_2 and 0.81 ml of NMM (7.2 mmol, 2.5 equiv.) were added. Solution was stirred at rt under N_2 overnight. The reaction was monitored on TLC (H:AcOEt 1:2). Solvent was partially removed by rv, the remaining oil was diluted with AcOEt, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. The crude product was further purified by dissolving it in boiling AcOEt and adding hexane till the solution got cloudy, then letting crystallize at -20°C. 963 mg of pure colourless crystals of methyl (2*S*)-3-(tosylamino)-2-[(1,3-thiazol-5-yl)carbonyl] propanoate (**66**) (2.51 mmol, 87%) could be isolated.

Melting temperature: 161.8 – 162.7°C
R_f(H/AcOEt 1:2): 0.18
$[\alpha]_D^{20}$ ($CHCl_3$): - 10.7



66

^{1}H-NMR (300 MHz, $CDCl_3$): 8.74 (*d*, ^{4}J = 2.1, 1 H, Thiazol C^2-H); 8.17 (*d*, ^{3}J = 8.6, 1 H, Amid-NH); 8.15 (*d*, ^{4}J = 2.1, 1 H, Thiazol C^5-H); 7.72 (*d*, ^{3}J = 8.1, 2 H, arom. *o*-CH); 7.24 (*d*, ^{3}J = 8.1, 2 H, arom. *m*-CH); 5.82 (*t*, ^{3}J = 6.6, 1 H, Sulfonamid-NH); 4.82 (*dt*, ^{3}J = 7.7, ^{3}J = 4.9, 1 H NCH); 3.76 (*s*, 3 H, OCH_3); 3.52 – 3.48 (*m*, 2 H, CH_2); 2.38 (*s*, 3 H, Ts-CH_3).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 169.99 (*s*, 1 C, COOMe); 161.04 (*s*, 1 C, CONH); 152.98 (*d*, 1 C, Thiazole-C^2H); 149.87 (*s*, 1 C, Thiazol-C^5); 143.45 (*s*, 1 C, Ts-C-Me); 136.71 (*s*, 1 C, Ts-C-S); 129.67 (*d*, 2 C, Ts-*m*-CH); 127.00 (*d*, 2 C, Ts-*o*-CH); 124.11 (*d*, 1 C, Thiazole-C^4H); 52.95 (*q*, 1 C, OCH_3); 52.36 (*d*, 1 C, NCH); 44.49 (*t*, 1 C, NCH_2N); 21.46 (*q*, 1 C, Ts-CH_3).

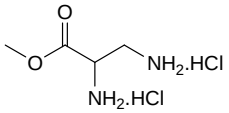
MS (ESI, MeOH + NaI): 406.1 (100, $[M + Na]^+$).

8.1 Target 1: [0.$_4$]{(2,4)1,3-thiazolo[0](2,4)-1*H*-1,3-imidazolo}phan (33)

8.1.1 Synthesis of azole amino acid "**A**"

8.1.1.1 Synthesis of **37**

In a oven dried flask equipped with a drying tube 0.503 g of 2,3-diaminopropionic acid hydrochloride (3.58 mmol) were suspended in 20 ml MeOH (*puriss. p.a.*) and cooled down to 0℃. 1.95 ml $SOCl_2$ (26.7 mmol, 7.5 equiv.) were added over 10 min through a dropping funnel. While stirring at 0℃ for 20 min the diaminoacid dissolved. The solution was then heated to 75℃ for 5 h [179]. The solvent was removed under reduced pressure, freezing it out in a cooling trap. The resulting colourless solid was dissolved in MeOH, filtered, the solvent was removed again and the substance dried in hv. 0.663 g of the pure methyl 2,3-diaminopropionate dihydrochloride (**37**, 3.473 mmol, 97%) were isolated.



37

^{1}H-NMR (300 MHz, CD_3OD): 4.49 (*dd*, ^3J = 7.9, ^3J = 5.6, 1 H, CH-NH_3); 3.94 (*s*, 3 H, OMe); 3.56 (*dd*, ^2J = 13.6, ^3J = 7.9, 1 H, CH_2NH_3); 3.48 (*dd*, ^2J = 13.6, ^3J = 5.6, 1 H, CH_2NH_3).

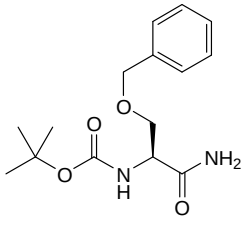
^{13}C-NMR (75.5 MHz, CD_3OD): 167.89 (*s*, 1 C, COOMe); 54.55 (*d*, 1 C, $CHNH_3$); 51.10 (*q*, 1 C, OCH_3); 39.47 (*t*, 1 C, CH_2NH_3).

8.1.1.2 Synthesis of **39**

14.34 g of **37** (48.55 mmol) were dissolved in 150 ml dioxane (*puriss. p.a.*), 2.5 ml pyridine (31 mmol, 0.66 equiv.), 15.487 g $(Boc)_2O$ (70.960 mmol, 1.5 equiv.) and 4.965 g $[NH_4][HCO_3]$ (62.80 mmol, 1.25 equiv.) were added and the mixture was stirred at rt under N_2 for 16 h. The reaction was monitored on TLC (H:AcOEt 2:1) staining with ninhydrin. The solvent was removed by rv, the remaining solid dissolved in AcOEt, washed with 0.1 M citric acid till all pyridine was removed (controlled by TLC), once with H_2O and once with brine. All aq. layers were back extracted with AcOEt, the comb. org. layers dried over $MgSO_4$, filtered and dried in rv and hv.
The remaining substance was dissolved in 30 ml CH_2Cl_2 and 450 ml hexane were added. The pure product was precipitated by removing a part of the CH_2Cl_2 in rv (45℃, 650 mbar). 13.20 g (44.85 mmol, 92%) of the colourless and solid L-Boc-Ser(OBn)-NH_2 (**39**) were isolated by filtration.

R_f (H/AcOEt 2 : 1): 0.1
$[\alpha]_D^{20}$ (Methanol): + 20.48
IR (KBr): 3389*s*; 3352*s*; 3289*m*, 3029*w*, 2969*w*; 2927*w*; 2864*w*; 2795*w*; 1686*s*; 1664*s*; 1524*s*; 1477*w*; 1454*m*; 1429*m*; 1393*w*; 1366*m*; 1315*m*; 1295*m*; 1249*m*; 1218*m*; 1170*m*; 1100*m*; 1049*m*; 1020*m*; 984*w*; 954*w*; 936*w*; 921*w*; 906*w*; 889*w*; 871*w*; 840*w*; 808*w*; 778*w*; 750*w*; 699*m*; 650*m*; 615*m*; 602*m*; 550*w*; 485*w*; 468*w*.

^{1}H-NMR (300 MHz, $CDCl_3$): 7.34 – 7.30 (*m*, 5 H, arom. CH); 6.42 (br. *s*, 1 H, NH); 5.39 (br. *s*, 2 H, NH_2); 4.61; 4.53 (2 *d*, ^2J = 11.7, 2 H, CH_2Ph); 4.30 (br. *s*, 1 H, NHC*H*CH_2); 3.92 (*dd*, ^2J = 9.2, ^3J = 3.8, 1 H, NHCHC*H*$_2$); 3.58 (*dd*, ^2J = 9.2, ^3J = 6.9, 1 H, NHCHC*H*$_2$); 1.45 (*s*, 9 H, $C(CH_3)_3$).



39

^{13}C-NMR (75.5 MHz, $CDCl_3$): 173.06 (*s*, 1 C, Amide-C); 155.46 (*s*, 1 C, Carbamate); 137.31 (*s*, 1 C, arom. C); 128.52; 127.97; 127.81 (3 *d*, 3 C, arom. CH); 80.23 (*s*, 1 C, CMe_3); 73.59 (*t*, 1 C, OCH_2Ph); 69.71 (*t*, 1 C, OCH_2CH); 53.58 (*d*, 1 C, $CHCH_2$); 28.23 (*q*, 3 C, $C(CH_3)_3$).

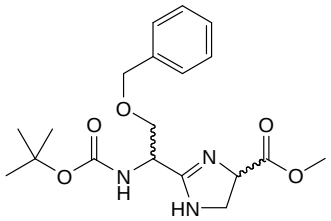
MS (ESI, MeOH/$CHCl_3$ 1:3 + NaI): 317.3 (100, $[M + Na]^+$); 217.1 (17, $[M - COOCMe_3 + H + Na]^+$).

8.1.1.3 Synthesis of **41**

In a 250 ml two necked flask 2.93 g of **39** (9.94 mmol) were dissolved in ca. 100 ml CH_2Cl_2 (*purum*) under Ar. 3.01 g of $CaCO_3$ (30.1 mmol, 3 equiv.) and 2.10 g Et_3OBF_4 (11.0 mmol, 1.1 equiv.) were added and the suspension was stirred at rt.Reaction was monitored by TLC (H:AcOEt 1:1). After 9 h, the solution containing intermediate **40** was filtered over 2 cm SiO_2 and diluted with CH_2Cl_2 to 200 ml.
2.02 g of **37** (10.6 mmol, 1.05 equiv.) were suspended in 150 ml CH_2Cl_2. 3.5 ml Et_3N (25 mmol, 2.5 equiv.) were added. The solution of **40** was added during 2 h. The suspension was then stirred overnight at rt under N_2. The reaction was monitored on TLC (H:AcOEt 1:1). The solution was then washed four times with 0.1 M citric acid, the comb. aq. layers were basified with sat. $NaHCO_3$ and back extracted three times with AcOEt. The org. layers were washed with brine, combined, dried over $MgSO_4$, filtered and the solvent removed in rv and hv. 2.33 g of the quite sufficiently pure methyl 2-{2-(benzyloxy)-1-[(*tert*-butoxycarbonyl)amino]}-1*H*-imidazoline-4-carboxylate (**41**) (6.17 mmol, 62% in respect to **39**) were isolated.

R_f(H/AcOEt 1:1): 0.1

^{1}H-NMR (300 MHz, $CDCl_3$): 7.35 – 7.32 (*m*, 5 H, arom. CH); 5.58 (br. *d*, ^3J = 13.1, 1 H, Carbamat-NH); 4.62 – 4.39; 3.92 – 3.81; 3.69 – 3.59 (3 *m*, 8 H, CH_2Ph, $NCHCH_2$, Imidazoline-$CHCH_2$); 3.75; 3.74 (2 *s*, 3 H, OMe (two diasteromers)); 1.44 (*s*, 9 H, $OC(CH_3)_3$).



41

^{13}C-NMR (75.5 MHz, $CDCl_3$): 173.20; 173.09 (2 *s*, COO (two diastereoisomers)); 166.939; 166.743 (2 *s*, 1 C, CNN (two diastereoisomers)); 155.32 (*s*, 1 C, Carbamat-CO); 137.54 (*s*, 1 C, arom. C); 128.29; 127.65; 127.00; 127.56 (4 *d*, 5 C, arom. CH (once diastereoisomers resolved)); 79.68 (*s*, 1 C, $OCMe_3$); 73.21; 73.15 (2 *t*, 1 C, OCH_2Ph (two diastereoisomers)); 70.68; 70.46 (2 br. *t*, 2 C, 2 CH_2); 61.78 (br. *d*, 1 C, CONHCH); 52.20 (*q*, 1 C, OCH_3); 49.08 (*d*, 1 C, CHNH); 28.17 (*q*, 3 C, $C(CH_3)_3$).

MS (ESI, MeOH/MeCN 1:1 + 0.1% HCOOH): 378.2 (100, $[M + H]^+$); 322.2 (20, $[M - CMe_3 + 2\ H]^+$).

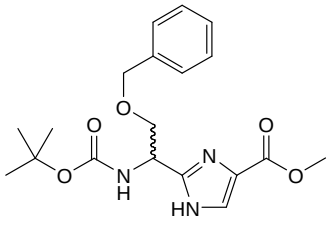
8.1.1.4 Synthesis of **42**

246 mg of **41** (652 µmol) were dissolved in 10 ml CH_2Cl_2 (tech.) and 636 mg of the 90% MnO_2 (6.58 mmol, 10 equiv.) were added[182]. The suspension was stirred at rt under N_2. The reaction was monitored by TLC (H:AcOEt 1:1). After 72 h another 634 mg of MnO_2 (6.58 mmol, 10 equiv.) were added. The suspension was further stirred for 24 h, then filtered over celite and chromatographically purified (H:AcOEt 2:1). 84

mg (305 µmol, 47%) of the pure product methyl 2-{2-(benzyloxy)-1-[(*tert*-butoxycarbonyl)amino] ethyl}-1*H*-imidazole-4-carboxylate (**42**) could be isolated.

Melting temperature: 156.2 – 157.9°C
R_f(H/AcOEt 1:1): 0.35

^{1}H-NMR (300 MHz, $CDCl_3$): 7.63 (*s*, 1 H, Imidazole-CH); 7.36 – 7.26 (*m*, 5 H, arom. CH); 5.64 (*d*, ^3J = 6.6, 1 H, NHCH); 4.97 (*dd*, ^3J = 12.8, ^3J = 6.2, 1 H, NCH); 4.58; 4.53 (2 *d*, ^2J = 11.9, 2 H, OCH_2Ph); 4.00 (br. *t*, *J* = 7.5, 1 H, Imidazole-NH); 3.87 (*s*, 3 H, OCH_3); 3.86 – 3.71 (*m*, 2 H, CH_2); 1.43 (*s*, 9 H, $OC(CH_3)_3$).


42

^{13}C-NMR (75.5 MHz, $CDCl_3$):

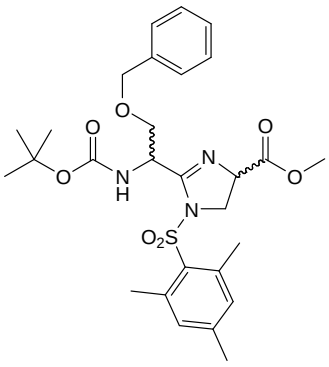
X-ray crystallography: A nice crystal of **42**, sutable for x-ray analysis, could be obtained by isothermal destillation of pentane into a solution of **42** in dioxane.

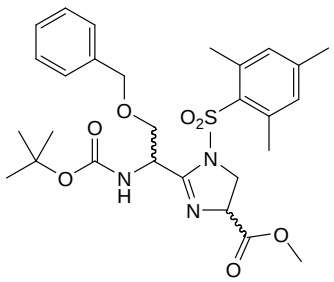
8.1.1.5 Synthesis of of **43** (mixture)

2.27 g of **41** (6.01 mmol) were dissolved in 250 ml CH_2Cl_2 under N_2 at rt. 1.7 ml of Et_3N (12 mmol, 2 equiv.) and 2.63 g of mesitylenesulfonylchloride (12.0 mmol, 2 equiv.) were added and the solution was stirred for 24 h. The reaction was monitored on TLC (H:EE 1:1). The solvent was partially removed in rv, the remaining solution diluted with TBME, washed three times with 0.1 M citric acid, twice with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME, the combined org. layers dried over $MgSO_4$, filtered and the solvent was removed in rv. The product was chromatographically purified on SiO_2 (H:AcOEt 5:1 to 2:1). 2.67 g of an otherwise pure mixture of the two isomers methyl 2-{2-(benzyloxy)-1-[(*tert*-butoxycarbonyl)amino]ethyl}-1-mesityl-1*H*-imidazoline-4-carboxylate (**43a**) (4.77 mmol, 79%) were isolated.

R_f(H/AcOEt 1:1): 0.5

^{1}H-NMR (300 MHz, $CDCl_3$): 7.33 – 7.25 (*m*, 5 H, arom. Ph-CH); 6.98; 6.96 (2 *s*, 2 H, arom. Mts-CH); 5.56 – 5.66 (*m*, 1 H, NH); 5.33 – 5.23 (*m*, 1 H, NCH); 4.69 – 4.37 (*m*, 4 H, OCH_2Ph, Imidazoline-CH_2); 3.81 – 3.55 (*m*, 6 H, OCH_3, OCH_2, Imidazoline-CH); 2.63; 2.62 (2 *s*, 6 H, 2 Mts-*ortho*-CH_3); 2.31; 2.30 (2 *s*, 3 H, Mts-*para*-CH_3); 1.43 (*s*, 9 H, $OC(CH_3)_3$).

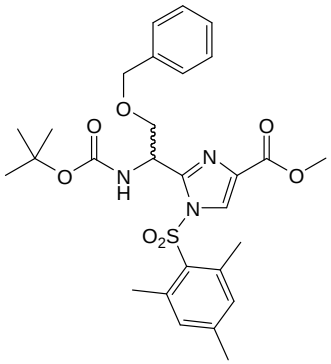

43a


43b

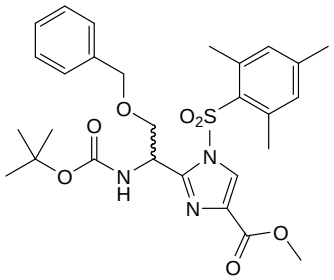
8.1.1.6 Synthesis of **35**

2.67 g of **43** (4.77 mmol) were dissolved in 150 ml CH_2Cl_2 under N_2. 0.80 ml of DBU (5.2 mmol, 1.1 equiv.) and 0.57 ml of $BrCCl_3$ (5.7 mmol, 1.2 equiv.) were added[182] at rt and the solution was stirred overnight. Reaction was monitored on TLC (H:AcOEt 1:1).
The CH_2Cl_2 was removed in rv, the remaining oily solution diluted with AcOEt, washed three times with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and the solvent removed in rv. The product was chromatographically purified on SiO_2 (H:AcOEt 4:1). 1.96 g of the pure product methyl 2-{2-(benzyloxy)-1-[(*tert*-butoxycarbonyl)amino]ethyl}-1-mesityl-1*H*-imidazole-4-carboxylate (**35a**) (3.51 mmol, 74%) could be isolated as a colourless foam.

R_f(H/AcOEt 1:1): 0.6



35a



35b

^{1}H-NMR (300 MHz, $CDCl_3$): 7.90 (*s*, 1 H, Imidazole-H); 7.31 – 7.24 (*m*, 3 H., arom. *ortho*/*para* CH); 7.17 – 7.14 (*m*, 2 H, arom. *m* CH); 6.95 (*s*, 2 H, arom. Mts-CH); 5.41 (*d*, ^3J = 8.8, 1 H, NH); 5.29 – 5.22 (*m*, 1 H, NCH); 4.40; 4.27 (2 *d*, ^2J = 12.1, 2 H, CH_2Ph); 3.89 (*s*, 3 H, OCH_3); 3.59 – 3.55 (*m*, 2 H, CH_2O); 2.53 (*s*, 6 H, 2 Mts-*ortho*-CH_3); 2.29 (*s*, 3 H, Mts-*para*-CH_3); 1.38 (*s*, 9 H, $C(CH_3)_3$).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 162.18 (*s*, 1 C, COO); 154.47 (*s*, 1 C, OCON); 148.40 (*s*, 1 C, CNN); 145.78 (*s*, 1 C, Mts *p*-C); 140.77 (*s*, 2 C, Mts *o*-C); 137.68 (*s*, 1 C, Ph *o*-C); 132.70 (*d*, 3 C, 2 Mts *m*-CH and Ph *p*-CH); 131.39; 130.85 (2 *s*, 2 C, Mts *o*-C, Im NC); 128.13; 127.44 (2 *d*, 4 C, Ph *o*/*m*-CH); 125.08 (*d*, 1 C, Im CH); 79.50 (*s*, 1 C, $OCMe_3$); 72.71; 71.31 (2 *t*, 2 C, 2 OCH_2); 52.06 (*q*, 1 C, OCH_3); 47.60 (*d*, 1 C, NHCH); 28.13 (*q*, 3 C, $C(CH_3)_3$); 22.48 (*q*, 2 C, Mts *o*-CH_3); 21.08 (*q*, 1 C, Mts *p*-CH_3).

MS (ESI, MeOH): 596.2 (15, $[M + K]^+$); 585.2 (5); 580.4 (66, $[M + Na]^+$); 558.4 (100, $[M + H]^+$).

8.1.2. Synthesis of azole amino acid "**B**"

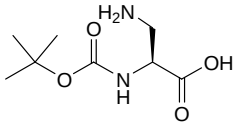
8.1.2.1 Synthesis of **50**

The synthesis of the compounds **46**, **47** and **50** is described in section 8.2.2.1, 8.2.2.2 and 8.2.2.4.

8.1.2.2 Synthesis of **48**

34.89 g of Boc-Asn-OH (150.2 mmol) were suspended in 180 ml AcOEt, 180 ml MeCN and 90 ml H_2O under N_2 and cooled down to 14 – 16°C. The suspension was mechanically stirred and 58.06 g of PIDA (180.3 mmol, 1.2 equiv.) were added[183].

After some minutes everything dissolved. The solution was kept at 14 – 16°C for 30 min and then slowly warmed up to rt. A new colourless precipitate formed. The suspension was stirred overnight, then cooled down to 0°C and filtered. The colourless solid was washed with little AcOEt and dried in hv at 60°C. 20.19 g 3-amino-2-[(*tert*-butoxycarbonyl)amino]propionic acid (Boc-DAP-OH, **48**) (98.85 mmol, 66%) were isolated.



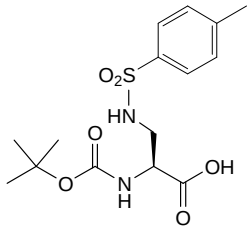
48

^{1}H-NMR (300 MHz, MeOD): 4.88 (br. *s*, 3 H exchangabel NH_2 and OH); 4.07 (br. *t*, ^3J = 6.0, 1 H, NCH); 3.17 (br. *d*, ^3J = 5.7, 2 H, CH_2); 1.46 (*s*, 9 H, $OC(CH_3)_3$).

^{13}C-NMR (75.5 MHz, MeOD): 174.94 (*s*, 1 C, COOH); 157.96 (*s*, 1 C, Carbamat-CO); 80.89 (*s*, 1 C, $OCMe_3$); 53.90 (*d*, 1 C, NCH); 43.03 (*t*, 1 C, CH_2); 28.68 (*q*, 3 C, $OC(CH_3)_3$).

8.1.2.3 Synthesis of **49**

5.02 g of **48** (24.6 mmol) were dissolved in 200 ml MeCN, 50 ml H_2O and 50 ml $NaHCO_3$. The solution was cooled down to 0°C and vigorously stirred using a mechanical stirrer. 4.90 g of TsCl (25.7 mmol, 1.05 equiv.) were added[184]. The solution was slowly warmed up to rt and reaction monitored on TLC (CH_2Cl_2:MeOH 5:1 staining with ninhydrin). After 6 h a part of the solvent was removed in rv and the remaining colourless mixture was diluted with 0.1 M citric acid. A new white oily precipitate formed, which was extracted three times with CH_2Cl_2. The org. layers were washed with brine, dried over $MgSO_4$, filtered and the solvent removed in rv and hv. 7.56 g (21.1 mmol, 86%) of (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(4-toluenesulfonylamino)propionic acid (Boc-DAP(NTs)-OH, **49**) were isolated as a solid colourless foam.



49

^{1}H-NMR (300 MHz, $CDCl_3$): 7.73 (*d*, ^3J = 8.5, 2 H, Tosyl-*o*-CH); 7.28 (*d*, ^3J = 8.5, 2 H, Tosyl-*m*-CH); 5.84 (br. *t*, ^3J = 5.9, 1 H, N*H*CH_2); 5.76 (br. *d*, ^3J = 7.2, 1 H, N*H*CH); 4.35 (br. *s*, 1 H, NCH); 3.39 – 3.36 (*m*, 2 H, NCH_2); 2.41 (*s*, 3 H, Tosyl-CH_3); 1.45 (*s*, 9 H, $OC(CH_3)_3$).

^{13}C-NMR (75.5 MHz, DMSO): 171.73 (*s*, 1 C, COOH); 155.14 (*s*, 1 C, Carbamate-CO); 142.71 (*s*, 1 C, Ts-*p*-CMe); 137.14 (*s*, 1 C, Ts-*i*-C); 129.63; 126.50 (2 *d*, 4 C, Ts-CH); 78.25 (*s*, 1 C, $OCMe_3$); 53.54 (*d*, 1 C, NCH); 43.61 (*t*, 1 C, NCH_2); 28.09 (*q*, 3 C, $OC(CH_3)_3$); 20.93 (*q*, 1 C, Ts-CH_3).

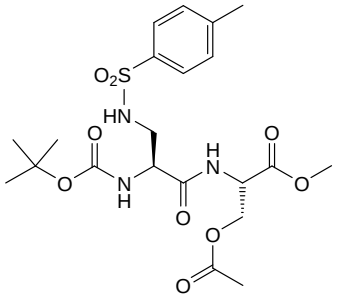
MS (ESI, $CHCl_3$/MeOH 3:1 + NaI): 739.2 (6, $[2M + Na]^+$); 567.3 (30); 381.1 (100, $[M + Na]^+$); 325.2 (21, $[M - C(CH_3)_3 + H + Na]^+$).

8.1.2.4 Synthesis of **51**

5.03 g of **49** (14.0 mmol) were dissolved in 80 ml CH_2Cl_2 (*purum*) under N_2, 3.9 ml of NMM (35 mmol, 2.5 equiv.) and 4.508 g of TBTU (14.04 mmol, 1 equiv.). 1 equiv of **79**, as described above, dissolved in 80 ml CH_2Cl_2 and 3.9 ml of NMM (35 mmol, 2.5 equiv.) were added. The reaction was monitored on TLC (H:AcOEt 1:1). The solution was stirred under N_2 overnight. A part of the solvent was removed by rv, the remaining solution diluted with TBME, washed once with 1 M aq. $NaHSO_4$, once with

aq. 0.1 M citric acid and once with brine. All aq. layers were back extracted with TBME, the comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. The isolated 9.09 g crude **51** were adsorbed to SiO_2 and chromatographically purified on SiO_2 (H:AcOEt 2:1 to 1:1). 4.78 g pure Boc-DAP(NTs)-Ser(OAc)-OMe (**51**) (9.53 mmol, 68%) were isolated.

R_f(H/AcOEt 1:1): 0.35


51

^{1}H-NMR (300 MHz, $CDCl_3$): 7.75 (*d*, ^3J = 8.3, 2 H, Ts-*o*-CH); 7.37 (br. *d*, ^3J = 6.2, 1 H, Amid-NH); 7.31 (*d*, ^3J = 8.3, 2 H, Ts-*m*-CH); 5.82 (br. *t*, ^3J = 5.9, 1 H, Ts-NH); 5.71 (br. *d*, ^3J = 5.7, 1 H, Carbamat-NH); 4.73 (*dt*, ^3J = 7.2; ^3J = 3.5, 1 H, *C*-terminal NCH); 4.59 (*dd*, ^2J = 11.7, ^3J = 3.2, 1 H, OCH_2); 4.38 (*dd*, ^2J = 11.7, ^3J = 3.5, 1 H, OCH_2); 4.30 – 4.25 (*m*, 1 H, *N*-terminal NCH); 3.79 (*s*, 3 H, OCH_3); 3.39 – 3.31; 3.22 – 3.12 (2 *m*, 2 H, NCH_2); 2.42 (*s*, 3 H, Ts-CH_3); 2.10 (*s*, 3 H, (CO)CH_3); 1.46 (*s*, 9 H, $OC(CH_3)_3$).

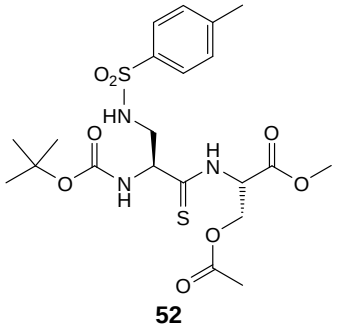
^{13}C-NMR (75.5 MHz, $CDCl_3$): 171.24; 170.65; 169.54 (3 *s*, 3 C, 2 COO, 1 CONH); 155.75 (*s*, 1 C, Carbamat-CO); 143.70 (*s*, Ts-*C*Me); 136.59 (*s*, 1 C, Ts-S-C); 129.85 (*d*, 2 C, Ts-*o*-CH); 127.10 (*d*, 2 C, Ts-*m*-CH); 80.77 (*s*, 1 C, $OCMe_3$); 63.03 (*t*, 1 C, OCH_2); 53.74; 53.04 (2 *d*, 2 C, 2 NCH); 52.34 (*q*, 1 C, OCH_3); 44.49 (*t*, 1 C, NCH_2); 28.28 (*q*, 3 C, $OC(CH_3)_3$); 21.52 (*q*, 1 C, Ts-CH_3); 20.72 (*q*, 1 C, (CO)CH_3).

MS (ESI, MEOH/CH_2Cl_2 3:1 + NaI): 524.3 (100, $[M + Na]^+$); 468.2 (10, $[M – CMe_3 + H + Na]^+$); 424.3 (6, $[M – CO_2CMe_3 + H + Na]^+$).

8.1.2.5 Synthesis of **52**

0.32 g of **51** (0.65 mmol) were dissolved in 25 ml toluene and 0.13 g of Lawesson reagent (0.32 mmol, 0.5 equiv.) were added. The solution was heated under reflux and N_2 for two hours. Reaction was monitored by TLC (H:AcOEt 1:1)[2]. The solution was diluted with TBME, washed once with 0.1 M citric acid, once with sat. $NaHCO_3$ and twice with brine. All aq. layers were back extracted with TBME, the comb. org. layers were dried over $MgSO_4$, filtered and the solvent was removed in rv. 0.38 g of the crude product were isolated as a yellowish, smelling oil. The substance was adsorbed at SiO_2 and chromatographically purified on SiO_2 (H:AcOEt 3:1). 0.27 g of Boc-ThioDAP(NTs)-Ser(OAc)-OMe (**52**) (mainly the open form, max. 0.52 mmol, 80%) could be isolated and were directly used for ring-closure and oxidation reaction.

R_f(H/AcOEt 2:1): 0.4; R_f(H/AcOEt 1:1): 0.75


52

^{1}H-NMR (300 MHz, $CDCl_3$): 8.90 (br. *d*, ^3J = 4.6, 1 H, Thioamid-NH); 7.75 (*d*, ^3J = 8.1, 2 H, Ts-*o*-CH); 7.32 (*d*, ^3J = 8.1, 2 H, Ts-*m*-CH); 5.84 (br. *s*, 1 H, Carbamat-NH); 5.59 (br. *t*, ^3J = 7.3, 1 H, Ts-NH); 5.22 (*dt*, ^3J = 6.9; ^3J = 3.1, 1 H, *C*-terminal NCH); 5.01 (*dd*, ^2J = 11.7, ^3J = 3.1, 1 H, OCH_2); 4.61 – 4.56 (*m*, 1 H, *N*-terminal NCH); 4.40 (*dd*, ^2J = 11.7,

[2] Longer reaction time favours the formation of the in principle desired oxazoline **52**, the subsequent reaction does not depend on the nature of **52** and yield seems to be better if the ring is not already formed.

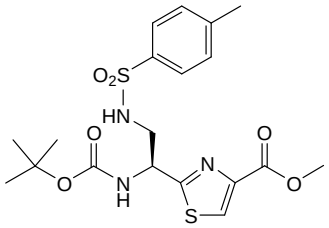
^3J = 2.7, 1 H, OCH_2); 3.85 (*s*, 3 H, OCH_3); 3.59 – 3.51; 3.25 – 3.16 (2 *m*, 2 H, NCH_2); 2.43 (*s*, 3 H, Ts-CH_3); 2.15 (*s*, 3 H, $(CO)CH_3$); 1.45 (*s*, 9 H, $OC(CH_3)_3$).

8.1.2.6 Synthesis of Boc-TDAP(NTs)-OMe (**44**)

269 mg of **52** (0.520 mmol) were dissolved in 20 ml CH_2Cl_2, 275 µl of DBU (1.82 mmol, 3.5 equiv.) and 62 µl of $BrCCl_3$ (0.624 mmol, 1.2 equiv.) were added and the solution was stirred at rt under N_2 for 3 h (monitored on TLC, H:AcOEt 1:1). The brown solution was then diluted with TBME, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME, the comb. org. layers were dried over $MgSO_4$, filtered and solvent was removed in rv. 194 mg of the crude product were isolated.
The oil was adsorbed at SiO_2 and chromatographically purified on SiO_2 (H:AcOEt 2:1). 181 mg (0.397 mmol, 61% in relation to **51**) of the pure methyl 2-{(1*S*)-1-[(*tert*-butoxycarbonyl)amino]-2-(tosylamino)ethyl}-1,3-thiazole-4-carboxylate (Boc-TDAP(NTs)-OMe, **44**) could be isolated.

R_f(H/AcOEt 1:1): 0.35

^{1}H-NMR (300 MHz, $CDCl_3$): 8.08 (*s*, 1 H, Thiazole-CH); 7.72 (*d*, ^3J = 8.1, 2 H, Ts-*o*-CH); 7.29 (*d*, ^3J = 8.1, 2 H, Ts-*m*-CH); 5.94 (br. *d*, ^3J = 6.7, 1 H, Carbamat-NH); 5.69 (br. *t*, ^3J = 6.8, 1 H, Ts-NH); 5.08 (*dt*, ^3J = 7.9, ^3J = 4.4, 1 H, NCH); 3.90 (*s*, 3 H, OCH_3); 3.56 – 3.44 (*m*, 2 H, CH_2); 2.42 (*s*, 3 H, Ts-CH_3); 1.46 (*s*, 9 H, $OC(CH_3)_3$).



44

^{13}C-NMR (75.5 MHz, $CDCl_3$): 171.86 (*s*, 1 C, Thiazol-CNS); 161.53 (*s*, 1 C, COOMe); 155.36 (*s*, 1 C, Carbamat-CO); 146.62 (*s*, Thiazole-*C*-COOMe); 143.71 (*s*, 1 C, Ts-C-Me); 136.64 (*s*, 1 C, Ts-S-C); 129.85 (*d*, 2 C, Ts-*o*-CH); 128.34 (*d*, 1 C, Thiazole-CH); 127.08 (*d*, 2 C, Ts-*m*-CH); 80.75 (*s*, 1 C, $OCMe_3$); 52.43 (*q*, 1 C, OCH_3); 52.39 (*d*, 1 C, NCH); 46.17 (*t*, 1 C, NCH_2); 28.30 (*q*, 3 C, $=C(CH_3)_3$); 21.52 (*q*, 1 C, Ts-CH_3).

MS (ESI, MeOH + NaI): 478.1 (100, $[M + Na]^+$); 422.1 (27, $[M - CMe_3 + H + Na]^+$); 378.1 (17, $[M - CO_2CMe_3 + H + Na]^+$).

8.1.3 Synthesis of tetramer Boc-[TDAP(NTs)]$_4$-OMe (**53**)

8.1.3.1 Synthesis of **54**

157 mg of **44** (345 µmol) were dissolved in 10 ml MeOH (*purum*) and 43.5 mg LiOH (1.82 mmol, 5 equiv.), dissolved in 10 ml H_2O, were added. The progress of ester hydrolysis was monitored on TLC (H:AcOEt 1:1). The solution was then acidified with 0.1 M aq. citric acid to pH 3 – 4 (formation of a white precipitate) and extracted twice with TBME. The org. layers were washed with brine, combined, dried over $MgSO_4$, filtered and dried in rv and hv. The acid **54** was isolated as colourless foam and directly used for peptide bond formation.

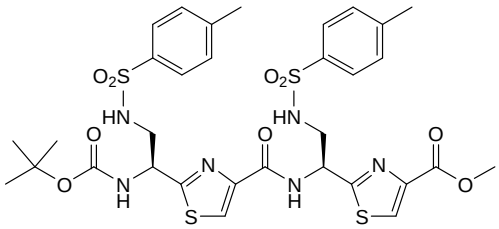
8.1.3.2 Synthesis of **55**

171 mg **44** (375 µmol) were dissolved in 2 ml CH_2Cl_2 (*purum*). A mixture of 2 ml CH_2Cl_2 and 2 ml TFA were added and the solution was stirred at rt for ~3 h under N_2, till TLC (H:AcOEt 1:1, staining with ninhydrin), showed no more **44**. The solvent was removed by rv freezing it out in a cooling trap. The oily residue was dissolved in MeOH, mixed with Amberlyst-A26 in its Cl^--form for 5 h, filtered and dried again in rv. The isolated ammonium salt **55** was directly used for peptide bond formation.

8.1.3.3 Synthesis of Boc-[TDAP(NTs)]$_2$-OMe (**56**)

152 mg of **54** (345 µmol) were dissolved in 10 ml distilled CH_2Cl_2, 240 µl of Et_3N (1.73 mmol, 5 equiv.), 112 mg of TBTU (345 µmol, 1 equiv.) and a solution of the 170 mg of **55** (362 µmol, 1 equiv.) in 10 ml distilled CH_2Cl_2 was added. Reaction was monitored on TLC (H:AcOEt 1:3). Reaction was stirred under N_2 overnight. A part of the solvent was removed by rv, the remaining solution diluted with TBME, washed twice with aq. 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME, the comb. org. layers dried over $MgSO_4$, filtered and dried in rv. The crude **56** was adsorbed at SiO_2 and chromatographically purified on SiO_2 (H:AcOEt 2:1 to 1:2). 261 mg pure Boc-[TDAP(NTs)]$_2$-OMe (**56**, 335 µmol, 97%) were isolated.

R_f(H/AcOEt 1:2): 0.15; R_f(H/AcOEt 1:3): 0.4



56

^{1}H-NMR (300 MHz, $CDCl_3$): 9.10 (br. *d*, ^3J = 5.1, 1 H, Amide-NH); 8.05 (*s*, *N*-terminal Thiazole-CH); 7.86 (br. *s*, *C*-terminal Thiazole-CH); 7.72 – 7.64 (*m*, 4 H, Ts-*o*-CH); 7.25 – 7.19 (*m*, 4 H, Ts-*m*-CH); 6.50; 6.43 (2 br. *s*, 2 H, Ts-NH); 6.16 (br. *d*, ^3J = 6.9, 1 H, Carbamate-NH); 5.45 (br. *d*, ^3J = 5.2, 1 H, NCH); 4.81 (br. *s*, 1 H, NCH); 3.82 (*s*, 3 H, OCH_3); 3.76 – 3.57; 3.25 – 2.97 (2 *m*, 4 H, 2 NCH_2); 2.42; 2.40 (2 *s*, 6 H, 2 Ts-CH_3); 1.44 (*s*, 9 H, $OC(CH_3)_3$).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 171.57; 171.25 (2 *s*, 2 C, Thiazole-NCS); 161.71; 161.64 (2 *s*, 2 C, COOMe, CONH); 155.31 (*s*, 1 C, Carbamate-CO); 148.27; 146.24 (2 *s*, 2 C, Thiazole-C^4); 143.69; 143.36 (2 *s*, 2 C, Ts-C-Me); 136.98; 136.56 (2 *s*, 2 C, Ts-C-S); 129.79; 129.68 (2 *d*, 4 C, Ts-*o*-C); 128.37 (*d*, 1 C, *N*-term. Thiazole-CH); 127.09; 127.00 (2 *d*, 4 C, Ts-*m*-CH); 125.40 (*d*, 1 C, *C*-term. Thiazole-CH); 80.46 (*s*, 1 C, $OCMe_3$); 52.54 (*q*, 1 C, OCH_3); 52.43; 52.22 (2 *d*, 2 C, NCH); 45.55; 45.03 (2 *t*, 2 C, NCH_2); 28.33 (*q*, 3 C, $OC(CH_3)_3$); 21.50 (*q*, 2 C, Ts-CH_3).

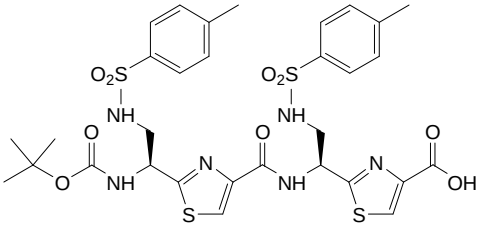
MS (ESI, MeOH + NaI): 801.3 (100, $[M + Na]^+$).

8.1.3.4 Synthesis of **141**

250 mg of **56** (321 µmol) were dissolved in 15 ml MeOH (*puriss. p.a.*). 47 mg of LiOH (2.0 mmol, 6 equiv.), dissolved in 5 ml H_2O, were added. The progress of the reaction was monitored on TLC (H:AcOEt 1:10 after little workup). A part of the solvent was removed in rv, the remaining oily solution was diluted with AcOEt, washed with 0.1 M aq. citric acid and 0.1 M citric acid in brine. The aq. layers were

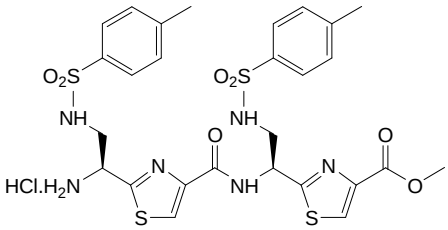
back extracted with AcOEt, the comb. org. layers dried over $MgSO_4$, filtered and the solvent removed in rv and hv. The crude **141** was directly used for peptide bond formation.

8.1.3.5 Synthesis of **142**



141

250 mg of **56** (321 µmol) were dissolved in 5 ml CH_2Cl_2 (*puriss. p.a.*). 15 ml of TFA were added and the solution was stirred at rt for ~6 h under N_2. Deprotection was monitored on TLC (H:AcOEt 1:10). The solvent was removed under reduced pressure freezing it out in a cooling trap. The oily residue was dissolved in MeOH, mixed with Amberlyst-A26 in its Cl^--form and stirred overnight, filtered and dried again in rv and hv. The crude **142**, isolated as colourless foam, was directly used for peptide bond formation.



142

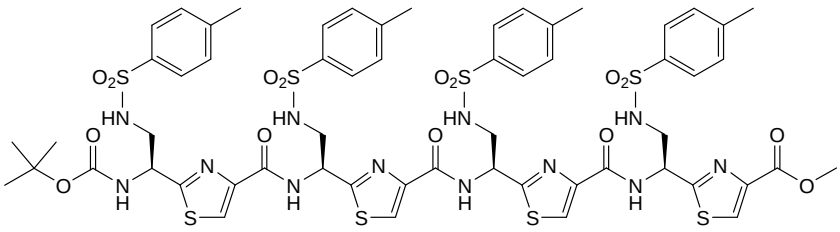
8.1.3.6 Synthesis of Boc-[TDAP(NTs)]$_4$-OMe (**53**)

321 µmol of **141** (1 equiv.) were dissolved in 20 ml dry DMF (solv. syst.) and 106 µl of NMM (963 µmol, 3 equiv.) were added.

231 µmol of **142** (1 equiv.) were dissolved in 20 ml dry DMF (solv. syst.) and 106 µl of NMM (963 µmol, 3 equiv.) were added.

251 mg of PyBOP (482 µmol, 1.5 equiv.) were added to the solution of **141**. One minute later the solution of **142** was added to the solution of **141**. The mixture was stirred under N_2 at rt overnight. Reaction was monitored on TLC (H:AcOEt 1:10).
The solvent was then partially removed in rv, the remaining oily substance diluted with AcOEt, washed once with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, the comb. org. layers were dried over $MgSO_4$, filtered and solvent was removed in rv. The isolated crude **53** was adsorbed at SiO_2 and chromatographically purified on SiO_2 (H:AcOEt 1:5 to 1:8). 335 mg pure Boc-[TDAP(NTs)]$_4$-OMe (**53**, 235 µmol, 73%) were isolated as a colourless foam.

R_f(H/AcOEt 1:10): 0.3
IR (KBr): 3324*s*; 2928*m*; 1718*s*; 1663*s*; 1598*m*; 1538*s*; 1495*s*; 1453*m*; 1368*m*; 1327*s*; 1247*s*; 1158*s*; 1093*s*; 1018*w*; 815*m*; 760*m*; 706*m*; 663*m*; 551*s*.



53

^{1}*H-NMR (300 MHz, DMSO)*: 9.01 – 8.93 (*m*, 3 H, Amide-NH); 8.45; 8.27; 8.25; 8.24 (4 *s*, Thiazole-CH); 7.97 – 7.94 (*m*, 3 H, Ts-NH); 7.77 (*t*, ^3J = 6.1, 1 H, Ts-NH); 7.69 – 7.64 (*m*, 8 H, Ts-*o*-CH); 7.60 (*d*, ^3J = 7.6, 1 H, Carbamate-NH); 7.36 – 7.26 (*m*, 8 H, Ts-*m*-CH); 5.45 – 5.41 (*m*, 3 H, NCH); 4.91 (br. *s*, 1 H, NCH); 3.83 (*s*, 3 H, OCH_3); 3.67 – 3.58; 3.55 – 3.38; 3.27 – 3.16 (3 *m*, 8 H, 4 NCH_2); 2.36; 2.33; 2.31; 2.30 (4 *s*, 12 H, 4 Ts-CH_3); 1.41 (*s*, 9 H, $OC(CH_3)_3$).

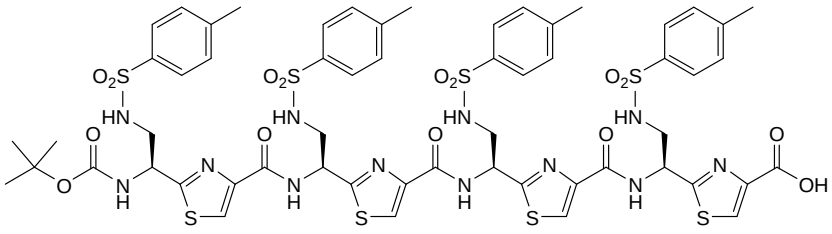
^{13}C-NMR (100 MHz, DMSO): 172.32; 170.87; 170.73 (3 *s*, 4 C, Thiazole-NCS); 161.08; 160.58; 160.46 (3 *s*, 4 C, COOMe, 3 CONH); 155.09 (*s*, 1 C, Carbamat-CO); 148.76; 148.63; 148.60; 145.37 (4 *s*, 4 C, Thiazole-C^4); 142.76; 142.66; 142.63 (3 *s*, 4 C, Ts-C-Me); 137.60; 137.31 (2 *s*, 4 C, Ts-C-S); 129.61; 129.54 (2 *d*, 8 C, Ts-*o*-CH); 126.50; 126.42 (2 *d*, 8 C, Ts-*m*-CH); 125.52; 125.33 (2 *d*, 4 C, Thiazole-CH); 78.91 (*s*, 1 C, $OCMe_3$); 52.95 (*d*, 1 C, NCH); 51.99 (*q*, 1 C, OCH_3); 51.67; 51.51 (2 *d*, 3 C, NCH); 45.49; 45.17; 44.95 (3 *t*, 4 C, NCH_2); 28.11 (*q*, 3 C, $OC(CH_3)_3$); 20.94; 20.90 (2 *q*, 4 C, Ts-CH_3).

MS (ESI, MeOH + NaI): 1447.4 (100, [*M* + Na]$^+$); 1347.1 (6, [*M* – CO_2CMe_3 + H + Na]$^+$).

8.1.4 Macrolactamization to **58**

8.1.4.1 Synthesis of **143**

328 mg of **53** (230 µmol) were dissolved in 40 ml hot MeOH (*puriss. p.a.*) and 33 mg of LiOH (1.4 mmol, 6 equiv.), dissolved in 20 ml H_2O, were added (c = 4 mM). The reaction was monitored on TLC (H:AcOEt 1:10) or better with HPLC. A part of the solvent was removed in rv, the remaining suspension was diluted with AcOEt, washed with 0.1 M citric acid and 0.1 M citric acid in brine. The org. layers were dried over $MgSO_4$, filtered and the solvent was removed in rv. The crude material **143** was isolated as a colourless solid and directly submitted to deprotection on the *N*-terminus.



143

8.1.4.2 Synthesis of **57**

Material **143** was dissolved in a mixture of 10 ml CH_2Cl_2 (*puriss. p.a.*) and TFA. The solution was stirred at rt under N_2. Reaction was monitored by HPLC. Completion was observed after about 5 h. The solvent was removed under reduced pressure, freezing it out in a cooling trap. The oily residue was dissolved in MeOH and some of the anion exchange resin Amberlyst-A26 in its Cl^--form was added. The mixture was stirred overnight, the resin was then filtered off and the solvent removed in rv an hv. The crude product **106,** a yellowish foam, was directly used for macrolactamisation.

8.1.4.3 Synthesis of **58**

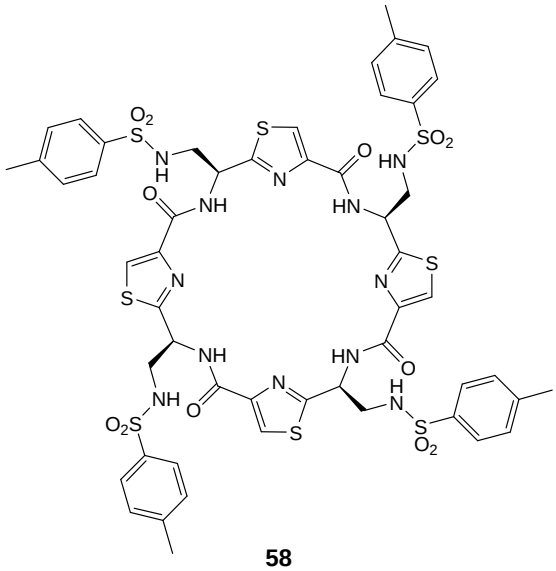
230 µmol of **57** were dissolved in 150 ml DMF (solv. syst., c = 1.5 mM). 0.25 ml of NMM (2.3 mmol, 10 equiv.) and 599 mg of PyBOP (1.15 mmol, 5 equiv.) were added and the solution was stirred at rt under N_2 overnight. Reaction was monitored on TLC ($CHCl_3$:MeOH 10:1) or better with HPLC. The solvent was partially removed, the remaining oil diluted with AcOEt, washed with 0.1 M citric acid and brine. All aq. layers were back extracted with AcOEt, the comb. org. layers were dried over

$MgSO_4$, filtered and the solvent removed in rv. The crude material was adsorbed at SiO_2 and chromatographically purified on SiO_2 (AcOEt:MeOH 15:1) .
126 mg of the pure and colourless solid (3*S*,11*S*,19*S*,27*S*) 3,11,19,27-tetra[(tosylamino)methyl]-1,9,17,25-tetroxo-2,10,18,26-tetraza[3.4](2,4)1,3-thiazolophan (**58**) (97 μmol, 42%) were isolated.

135 mg of a slightly less polar substance also showing nicely the right mass signal but a ^{1}H-NMR spectrum with some additional signals in the aromatic region were isolated. It seems to be a mixture of diastereoisomers.

Melting temperature: solidified foam, 132.2 – 132.6°C
R_f(AcOEt/MeOH 10:1): 0.35
IR (KBr): 3851*w*; 3747*w*; 3710*w*; 3448*s*; 2926*m*; 2864*m*; 1659*s*; 1542*s*; 1496*s*; 1454*m*; 1442*m*; 1384*m*; 1323*s*; 1256*m*; 1202*w*; 1158*s*; 1093*s*; 1071*m*; 1051*m*; 1039*m*; 1017*m*; 919*w*; 811*m*; 756*m*; 660*s*; 562*s*; 552*s*.

^{1}H-NMR (300 MHz, DMSO): 8.43 (br. *d*, ^3J = 8.0, 4 H, Amide-NH); 8.29 (*s*, 4 H, Thiazole-CH); 7.86 (br. *t*, ^3J = 6.1, 4 H, Ts-NH); 7.67 (*d*, ^3J = 8.2, 8 H, Ts-*o*-CH); 7.29 (*d*, ^3J = 8.2, 8 H, Ts-*m*-CH); 5.41 – 5.39 (*m*, 4 H, NCH); 3.51 – 3.33 (*m*, 8 H, 4 NCH_2); 2.33 (*s*, 12 H, 4 Ts-CH_3).



58

^{13}C-NMR (100 MHz, DMSO): 167.20 (*s*, 4 C, Thiazole-CNS); 159.72 (*s*, 4 C, Amide-CO); 147.65 (*s*, 4 C, Thiazole-C^4); 142.97 (*s*, 4 C, Ts-C-Me); 137.36 (*s*, 4 C, Ts-C-S); 129.75 (*d*, 8 C, Ts-*o*-CH); 126.46 (*d*, 8 C, Ts-*m*-CH); 126.24 (*d*, 4 C, Thiazole-CH); 49.61 (*d*, 4 C, NCH); 44.84 (*t*, 4 C, NCH_2); 21.00 (*q*, 4 C, Ts-CH_3).

MS (ESI, MeOH + NH_4-Acetate): 1315.3 (100, $[M + Na]^+$); 1293.3 (45, $[M + H]^+$); 1222.4 (33, $[M – NH_2Ts + H]^+$); 951.3 (12, $[M – 2\ NH_2Ts + H]^+$); 839.3 (7); 575.2 (10).

8.1.5 Investigations towards the synthesis of imidazole units

8.1.5.1 Reactions using model compounds

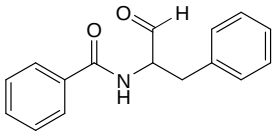
8.1.5.1.1 Reactions using **63**

a. Synthesis of aldehyde **67**

1.02 g of **63** (4.00 mmol) were suspended in 50 ml CH_2Cl_2 (*puriss. p.a.*) and cooled down to 0°C. 2.01 g of 97% Dess-Martin reagent (4.6 0 mmol, 1.15 equiv.) were added and the suspension was stirred overnight under N_2 slowly warming up to rt. Completion of the reaction was monitored on TLC (H:AcOEt 2:1). CH_2Cl_2 was removed by rv, the remaining oil diluted with AcOEt, washed once with 0.1 M citric acid, twice with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, the comb. org. layers dried over $MgSO_4$, filtered and dried in rv. The 1.254 g crude material were recrystallized from H:AcOEt 5:8. 616 mg (2.43 mmol,

61%) of the pure product *N*-(1-oxo-3-phenylpropan-2-yl)benzamide (**67**) could be isolated.

R_f(H/AcOEt 1:1): 0.8; R_f(H/AcOEt 1:1): 0.4



67

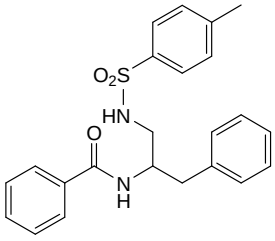
^{1}H-NMR (300 MHz, $CDCl_3$): 9.72 (*s*, 1 H, CHO); 7.74 – 7.71 (*m*, 2 H, arom. CH); 7.54 – 7.17 (*m*, 8 H, arom. CH); 6.75 (br. *d*, 1 H, NH); 4.91 (app. *q*, ^3J = 6.4, 1 H, NCH); 3.33 (*dd*, ^2J = 14.0, ^3J = 6.0, 1 H, CH_2Ph); 3.26 (*dd*, ^2J = 14.0, ^3J = 6.7, 1 H, CH_2Ph).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 198.77 (*d*, 1 C, CHO); 167.33 (*s*, 1 C, CONH); 135.60; 133.65 (2 *s*, 2 C, arom. C); 131.95; 129.40; 128.86; 128.69; 127.26; 127.06 (6 *d*, 10 C, arom. CH); 60.16 (*d*, 1 C, NCH); 35.19 (*t*, 1 C, CH_2Ph).

MS (ESI, CH_2Cl_2/MeOH 1:3 + NaI): 308.2 (100, [*M* + MeOH + Na]$^+$); 276.1 (6, [*M* + Na]$^+$).

b. Reductive amination of **67** using tosylamine

127 mg of **67** (501 µmol) were dissolved in 15 ml MeOH (*puriss. p.a.*) and 86 mg of $TsNH_2$ (502 µmol, 1 equiv.) were added and stirred at rt under N_2. After 45 min, the solution was cooled down to 0℃ and 35 mg of $NaBCNH_3$ (557 µmol, 1.1 equiv.) were added. The suspension was slowly let warm up to rt. TLC (H:AcOEt 2:1) shows a mixture of two substances with more or less the same R_f. Another equiv. of the reagents was added (86 mg $TsNH_2$ and 35 mg $NaBCNH_3$). The solution was heated up to 50℃ for 30 min, solvent was then removed by rv, the remaining oil diluted with AcOEt, washed once with sat. $NaHCO_3$ and twice with brine. The comb. aq. layers were dried over $MgSO_4$, filtered and dried in rv. 302 mg of a mixture were isolated. Product **69** was detected in ESI-MS.



69

MS (ESI, CH_2Cl_2/MeOH 1:4): 684.4 (11); 531.4 (53); 461.2 (56); 431.3 (100, [*M* + Na]$^+$); 429.2 (30); 308.1 (40); 278.2 (13, [M_{33} + Na]$^+$); 276.2 (12).

8.1.5.1.2 Reactions using **64**

a. Synthesis of the β-chloroamide **73** by chlorination of **64**

Method A: Using $SOCl_2$

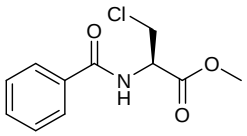
62 mg of **64** (0.28 mmol) were dissolved in 2 ml $SOCl_2$ and 2 µl DMF were added[189, 236]. The solution was heated up to reflux overnight. Solvent was then removed under reduced pressure freezing it out in a cooling trap. The remaining material was directly used for the next step.

Method B: Using LiCl and DAST

100 mg of **64** (448 µmol) were dissolved in 4 ml of a 0.5 M LiCl solution in DMF (86 mg LiCl (2.03 mmol) dried at 100℃ in hv for 2 h, 4 ml dry DMF (solv. syst.), c_{64} = 0.1 M) and cooled down to 0℃ in an ice bath. 88 µl of DAST (0.67 mmol, 1.5 equiv.) were added. The solution was let warm up to rt while stirring. The progress of the reaction was monitored on TLC (H:AcOEt 1:1). The solution was then diluted with AcOEt, washed with 0.1 M citric acid in brine, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. The crude product was chromatographically purified on SiO_2

(H:AcOEt 4:1 to 2:1). 82 mg of the pure methyl 2-(benzoylamino)-3-chloropropanoate **73** (0.34 mmol, 76%) were isolated as a colourless oil.

R_f(H/AcOEt 1:1): 0.32, R_f(H/AcOEt 3:1): 0.14


73

^{1}H-NMR (300 MHz, $CDCl_3$): 7.86 – 7.83 (*m*, 2 H, arom. *o*-CH); 7.58 – 7.45 (*m*, 3 H, arom. *m*-/*p*-CH); 7.02 (d, ^3J = 6.0, 1 H, NH); 5.21 (*dt*, ^3J = 7.3, ^3J = 3.0, 1 H, NCH); 4.10 (*dd*, ^2J = 11.4, ^3J = 2.8, 1 H, CH_2Cl); 4.04 (*dd*, ^2J = 11.4, ^3J = 3.1, 1 H, CH_2Cl); 3.87 (*s*, 3 H, OCH_3).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 169.40 (*s*, 1 C, COOMe); 167.03 (*s*, 1 C, CON); 133.40 (*s*, 1 C, arom. C); 132.09; 128.69; 127.17 (3 *d*, 5 C, arom. CH); 53.52 (*d*, 1 C, NCH); 53.13 (*q*, 1 C, OCH_3); 45.15(*t*, 1 C, CH_2Cl).

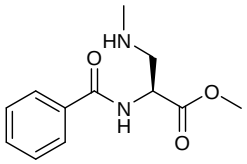
MS (ESI, MeOH + NaI): 316.1 (9); 264.0 (100, [*M* + Na]$^+$); 246.0 (6); 227.9 (12, [*M* – HCl + Na]$^+$).

b. Substitiution of chloride by an amine

b1. Using methylamine

15 mg of **73** (62 μmol) were dissolved in 1 ml DMF and 0.15 ml of the 8 M $MeNH_2$-solution were added (1.2 mmol, 20 equiv.). The solution was stirred at rt under N_2. Progress was monitored on TLC (H:AcOEt 1:1, then $CHCl_3$:MeOH 5:1). The solvent was removed and the product was purified on prep. TLC ($CHCl_3$:MeOH 5:1). 5 mg methyl 2-(benzoylamino)-3-(methylamino)propanoate (**76**) (21 μmol, 34%) were isolated.

R_f($CHCl_3$/MeOH 5:1): 0.23

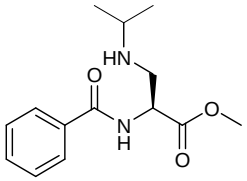

76

^{1}H-NMR (300 MHz, $CDCl_3$): 7.90 – 7.88 (*m*, 2 H, arom. CH); 7.58 – 7.45 (*m*, 3 H, arom. CH); 4.87 (*t*, ^3J = 5.9, 1 H, NCH); 3.80 (*s*, 3 H, OCH_3); 3.10 (*dd*, ^3J = 5.6, ^3J = 1.8, 2 H, CH_2); 2.47 (*s*, 3 H, NCH_3).

b2. Using isopropylamine

40 mg of **73** (0.17 mmol) were dissolved in 8 ml DMF (solv. syst.) under N_2 and cooled down to 0℃. 150 ml of isopropylamine (1.47 mmol, 10 equiv.) were added and the solution was let warm up to rt while stirring. The progress of the reaction was monitored on TLC ($CHCl_3$:MeOH 5:1). The solvent was removed in rv, the remaining material dissolved in AcOEt, washed once with sat. $NaHCO_3$, the aq. layer was back extracted, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. 36 mg of the quite pure methyl 2-(benzoylamino)-3-(isopropylamino)propanoate (**77**) (136 μmol, 82%) were isolated.

R_f($CHCl_3$/MeOH 5:1): 0.42


77

^{1}H-NMR (300 MHz, $CDCl_3$): 7.84 – 7.82 (*m*, 2 H, arom. *o*-CH); 7.52 – 7.41 (*m*, 3 H, arom. *p*/*m*-CH); 7.28 (br. *d*, ^3J = 5.8, 1 H, Amid-NH); 4.81 (*dt*, ^3J(NH) = 7.3, ^3J = 4.4, 1 H, NCHCO); 3.78 (*s*, 3 H, OCH_3); 3.18 (*dd*, ^2J = 12.6, ^3J = 4.4, 1 H, CH_2); 3.03 (*dd*, ^2J = 12.6, ^3J = 4.7, 1 H, CH_2); 2.81 (*sept*, ^3J = 6.2, 1 H, $CHMe_2$); 1.37

(br. *s*, 1 H, NH); 1.05; 1.04 (2 *d*, ^3J = 6.2, 6 H, $C(CH_3)_2$).

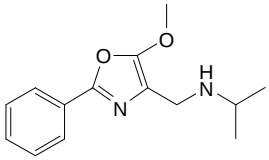
^{13}C-NMR (75.5 MHz, $CDCl_3$): 172.12 (*s*, 1 C, COO); 167.06 (*s*, 1 C, CON); 133.86 (*s*, 1 C, arom. C); 131.64 (*d*, 1 C, arom. *p*-CH); 128.49 (*d*, 2 C, arom. *o*-CH); 127.08 (*d*, 2 C, arom. *m*-CH); 52.77 (*d*, 1 C, amid-NCH); 52.42 (*q*, 1 C, OCH_3); 48.46 (*d*, 1 C, $NCHMe_2$); 47.62 (*t*, 1 C, NCH_2); 22.95; 22.89 (2 *q*, 2 C, 2 CH_3).

MS (ESI, MeOH +NaI): 287.1 (100, $[M + Na]^+$); 265.1 (11, $[M + H]^+$).

c. Synthesis of oxazole **144** by dehydration

15 mg of **77** (57 μmol) were dissolved in 2 ml MeCN in a dry flask with dry reflux condenser under N_2. 50 mg of P_2O_5 (352 μmol, 6 equiv.) were added and the suspension was heated to 60℃ for 2 h. The progress of the reaction was monitored on TLC ($CHCl_3$:MeOH 5:1). The solution was then diluted with AcOEt, washed with sat. $NaHCO_3$ and brine, the aq. layers were back extracted, the comb. org. layers dried over $MgSO_4$, filtered and the solvent removed in rv. 13 mg of the quite pure oxazole 4-isopropylamino-5-methoxy-2-phenyl-1,3-oxazole (**144**) (53 μmol, 93%) were isolated.

R_f($CHCl_3$/MeOH 5:1): 0.33



144

^{1}H-NMR (300 MHz, $CDCl_3$): 7.93 – 7.90 (*m*, 2 H, arom. CH); 7.45 – 7.38 (*m*, 3 H, arom. CH); 4.04 (*s*, 3 H, OCH_3); 3.69 (*s*, 2 H, NCH_2); 2.91 (*sept*, ^3J = 6.3, 1 H, $CHMe_2$); 2.35 (br. *s*, 1 H, NH); 1.13 (*d*, ^3J = 6.3, 6 H, $C(CH_3)_2$).

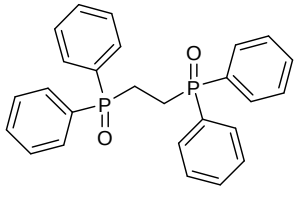
^{13}C-NMR (75.5 MHz, $CDCl_3$): 155.47; 152.36 (2 *s*, 2 C, Thiazole-C^2 / -C^5); 129.61; 128.65 (2 *d*, 3 C, arom. CH); 127.65 (*s*, 1 C, arom. C); 125.45 (*d*, 2 C, arom. CH); 114.90 (*s*, 1 C, Thiazole-C^4); 60.84 (*q*, 1 C, OCH_3); 47.77 (*d*, 1 C, NCH); 40.91 (*t*, 1 C, NCH_2); 22.50 (*q*, 2 C, *i*-Pr-CH_3).

MS (ESI, MeOH + 0.1% HCOOH): 269.0 (5, $[M + Na]^+$); 247.0 (51, $[M + H]^+$); 187.9 (64); 159.9 (100); 130.0 (7); 103.0 (46).

8.1.5.1.3 Reactions using **66**

a. Synthesis of **87**

1.00 g of 1,2-bis-(diphenylphosphino)ethan (2.51 mmol) were dissolved in a separating funnel in 100 ml CH_2Cl_2 (*tech.*). 10 ml aqueous 35% H_2O_2 solution was added and the mixture was shaken till no more gas evolution was observed[202]. The process was monitored on TLC (CH_2Cl_2). The org. layer was collected, washed twice with H_2O and the aqueous layers were back extracted. The remaining H_2O_2 was destroyed with catalytic MnO_2, the comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. The crude product was recrystallized from toluene to furnish 1.04 g (2.41 mmol, 96%) of the pure 1,1'-(1,2-ethanediyl)bis[1,1-diphenylphosphine oxide] (**87**).



87

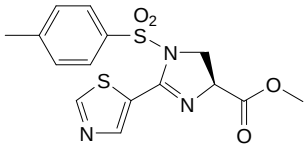
^{1}H-NMR (300 MHz, $CDCl_3$): 7.73 – 7.67 (*m*, 8 H, arom. CH); 7.54 – 7.28 (*m*, 12 H, arom. CH); 2.52 (*d*, $^3J_{P\text{-}H}$ = 2.6, 4 H, CH_2).

MS (ESI, MeOH + NaI): 453.2 (100, $[M + Na]^+$).

b. Kelly reaction for synthesis of imidazoline **85**

84 mg of **87** (0.20 mmol, 1.5 equiv.) were dissolved in 3 ml dry CH_2Cl_2 (*puriss. p.a.* o.m.s.), cooled down to 0°C under N_2 and 30 µl of Tf_2O (0.18 mmol, 1.5 equiv.) were added. Immediately a precipitate of the cyclic phosphoanhydride formed[182, 197, 202]. 15 min later 50 mg of **66** (0.13 mmol) were added[3]. After 15 min everything had dissolved again indicating good conversion. The solution was stirred overnight. Completion of the conversion was monitored on TLC (H:AcOEt 1:3). The solution was diluted with CH_2Cl_2, washed once with sat. $NaHCO_3$ and once with brine. Aq. layers were back extracted with CH_2Cl_2, comb. org. layers dried over $MgSO_4$, filtered and the solvent removed in rv. 135 mg of a mixture with **87** and methyl *S*-2-(1,3-thiazol-5-yl)-1-tosyl-1*H*-imidazoline-4-carboxylate (**85**) were isolated and directly used for the oxidation step.

R_f(H/AcOEt 1:3): 0.1



85

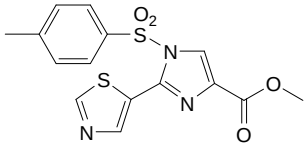
^{1}H-NMR (300 MHz, $CDCl_3$): 8.83 (*d*, ^4J = 2.0, 1 H, NC^{2}HS); 8.04 (*d*, ^4J = 2.0, 1 H, NC^{4}HC); 7.70 (*d*, ^3J = 8.2, 2 H, arom. *o*-CH); 7.31 (*d*, ^3J = 8.2, arom. *m*-CH); 4.65 (*dd*, ^3J = 8.9, ^3J = 10.3, 1 H, NCH); 4.27 – 4.24 (*m*, 2 H, CH_2); 3.72 (*s*, 3 H, OCH_3); 2.43 (*s*, 3 H, Ts-CH_3).

MS (ESI, MeOH + NaI): 579.2 (27, $[2\ M_{Ph3PO} + Na]^+$); 406.1 (12, $[M_{\mathbf{176}} + Na]^+$); 388.1 (100, $[M + Na]^+$); 366.1 (6, $[M + H]^+$); 301.1 (68, $[M_{Ph3PO} + Na]^+$); 279.2 (12, $[M_{Ph3PO} + H]^+$).

c. Synthesis of the imidazole **145**

135 mg of a mixture of **85** (max. 130 µmol) and **87** were dissolved in 5 ml CH_2Cl_2 under N_2 and cooled down to -10°C. 40 µl of DBU (0.26 mmol, 2 equiv.) and 20 µl of $BrCCl_3$ (0.20 mmol, 1.5 equiv.) were added. The solution was slowly let warm up to rt while stirring. After 90 min TLC (H:AcOEt 1:2) monitored complete conversion. The solution was diluted with CH_2Cl_2, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine, all aq. layers were back extracted with CH_2Cl_2, comb. org. layers dried over $MgSO_4$, filtered and the solvent removed in rv. The 95 mg of crude product were further purified over SiO_2 (H:AcOEt 1:1). 31 mg of the pure methyl 2-(1,3-thiazol-5-yl)-1-tosyl-1*H*-imidazole-4-carboxylate (**145**) (85 µmol, 66% in two steps) could be isolated.

R_f(H/AcOEt 1:2): 0.4



145

^{1}H-NMR (300 MHz, $CDCl_3$): 8.81 (*d*, ^4J = 2.3, 1 H, Thiazole-C^{2}H); 8.29 (*s*, 1 H, Imidazole-C^{5}H); 7.97 (*d*, ^4J = 2.3, 1 H, Thiazole-C^{5}H); 7.93 (*d*, ^3J = 8.3, 2 H, Ts-*o*-CH); 7.34 (*d*, ^3J = 8.3, Ts-*m*-CH); 3.92 (*s*, 3 H, OCH_3); 2.44 (*s*, 3 H, Ts-CH_3).

[3] The reaction was also performed following the original Kelly protocol[182, 197] using 3 equiv. Ph_3PO and 1.5 equiv. Tf_2O as described for the examples with **56** or **53** in section 8.1.5.1.4.

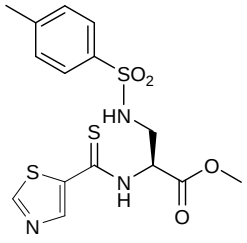
^{13}C-NMR (75.5 MHz, $CDCl_3$): 162.17 (*s*, 1 C, COOMe); 152.05 (*d*, 1 C, Thiazole-C^2H); 146.64; 144.39; 142.45; 134.20; 132.63 (5 *s*, Ts-*i*/*p*-C, Thiazole-C^4, Imidazole-C^2/C^4); 129.92 (*d*, 2 C, Ts-*m*-CH); 128.83 (*d*, 2 C, Ts-*o*-CH); 126.46 (*d*, 1 C, Imidazole-C^5H); 122.68 (*d*, 1 C, Thiazole-C^5H); 52.21 (*q*, 1 C, OCH_3); 21.81 (*q*, 1 C, Ts-CH_3).

MS (ESI, MeOH + NaI): 386.1 (100, $[M + Na]^+$); 364.2 (7, $[M + H]^+$).

d. Synthesis of the thioamide **84**

40 mg of **66** (0.10 mmol) were dissolved in 2 ml pyridine (*puriss. p.a.* o.m.s.). 46 mg of P_2S_5 (0.21 mmol, 2 equiv.) were added and the solution was heated up to reflux (oil bath at 150°C). After 2 h TLC (H:AcOEt 1:3) showed complete conversion. Solvent was removed under reduced pressure freezing it out in a cooling trap. The material was adsorbed at SiO_2 and the substance chromatographically purified on SiO_2 (H:AcOEt 2:1 to 1:1). 32 mg of the pure methyl 3-(tosylamino)-2-[(1,3-thiazol-5-yl)thiocarbonyl]propanate (**84**) (80 µmol, 77%) were isolated.

R_f(H/AcOEt 1:3): 0.6



84

^{1}H-NMR (300 MHz, $CDCl_3$): 9.87 (*d*, ^3J = 7.2, 1 H, Thioamide-NH); 8.77 (*d*, ^4J = 2.1, 1 H, Thiazol-C^2H); 8.38 (*d*, ^4J = 2.1, 1 H, Thiazol-C^4H); 7.70 (*d*, ^3J = 8.2, 2 H, 2 arom. *o*-CH); 7.23 (*d*, ^3J = 8.2, 2 H, 2 arom. *m*-CH); 5.39 (*dt*, $^3J_{NH}$ = 7.6, $^3J_{CH}$ = 4.4, 1 H, CH); 5.31 (*t*, ^3J = 6.8, 1 H, Ts-NH); 3.80 (*s*, 3 H, OCH_3); 3.72 (*ddd*, ^2J = 13.9, $^3J_{NH}$ = 6.5, $^3J_{CH}$ = 4.3, 1 H, CH_2); 3.59 (*ddd*, ^2J = 13.9, $^3J_{NH}$ = 7.1, $^3J_{CH}$ = 4.5, 1 H, CH_2); 2.38 (*s*, 3 H, Tosyl-CH_3).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 187.55 (*s*, 1 C, CSNH); 169.61 (*s*, 1 C, COOMe); 154.79 (*s*, 1 C, Thiazol-C^5); 152.75 (*d*, 1 C, Thiazol-C^2H); 143.89 (*s*, 1 arom. *i*-C); 136.75 (*s*, 1 arom. *p*-C); 129.97; 129.90; 127.31; 126.66 (4 *d*, 4 arom. CH); 126.27 (*d*, 1 C, Thiazol-C^4H); 57.16 (*q*, 1 C, OCH_3); 53.40 (*d*, 1 C, NCH); 43.65 (*t*, 1 C, NCH_2); 21.73 (*q*, 1 C, Tosyl-CH_3).

MS (ESI, MeOH + NaI): 422.0 (100, $[M + Na]^+$).

e. Sulphur abstraction and formation of imidazoline **85**

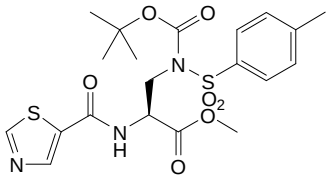
9 mg of **84** (0.02 mmol) were dissolved in 1 ml pyridine (*puriss. p.a.* o.m.s.). 6 mg of orange HgO (0.03 mmol, 1.2 equiv.) and 4 µl of Et_3N (0.03 mmol, 1.2 equiv.) were added[196] and the suspension was heated up to 100°C for 2 h. TLC (H:AcOEt 1:3) showed clean conversion. The suspension was diluted with AcOEt, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. 8 mg of the quite clean product **85** (0.02 mmol, 96%) were isolated.

f. Secundary protection of the β-tosylaminoamide

100 mg of **66** (261 µmol) were dissolved in 2 ml MeCN. 63 mg of Boc_2O (0.29 mmol, 1.1 equiv. and 5 mg of DMAP (0.04 mmol, cat. 0.1 equiv.) were added. The solution was stirred at rt under N_2. After 4 h TLC (1:3) confirmed complete conversion to a single product. The solution was diluted with AcOEt, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with AcOEt, comb. org. layers were dried over $MgSO_4$, filtered and solvent removed in rv. 126 mg of the

pure methyl 3-[(*t*-butyloxycarbonyl)tosylamino]-2-[(1,3-thiazol-5-yl) carbonyl]propanate (**82**) (261 µmol, 100%) could be isolated.

R_f(H/AcOEt 1:3): 0.55



82

^{1}H-NMR (300 MHz, $CDCl_3$): 8.78 (*d*, ^4J = 2.0, 1 H, Thiazole-C^2H); 8.20 (br. *d*, ^3J = 5.5, 1 H, NH); 8.19 (*d*, ^4J = 2.0, 1 H, Thiazole-C^5H); 7.78 (*d*, ^3J = 8.3, 2 H, Ts-*o*-CH); 7.23 (*d*, ^3J = 8.3, 2 H, Ts-*m*-CH); 5.17 (*dt*, $^3J_{CH2}$ = 7.8, $^3J_{NH}$ = 6.3, 1 H, NCH); 4.33 (*dd*, ^2J = 1.8, $^3J_{CH}$ = 7.8, 2 H, CH_2); 3.80 (*s*, 3 H, OCH_3); 2.40 (*s*, 3 H, Ts-CH_3); 1.34 (*s*, 9 H, $OC(CH_3)_3$).

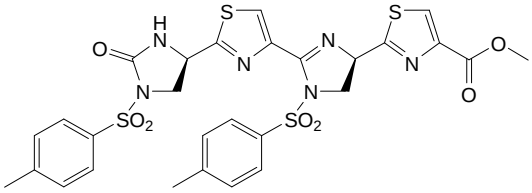
MS (ESI, MeOH + NaI): 606.2 (6, $[M - H + Boc + Na]^+$); 506.2 (100, $[M + Na]^+$); 406.1 (92, $[M - Boc + H + Na]^+$).

8.1.5.1.4 Reactions using building blocks **56** and **53**

a. Synthesis of **81**

In 1 ml dry (freshly distilled over CaH_2) CH_2Cl_2 214 mg of Ph_3PO (770 µmol, 6 equiv) were dissolved and cooled down to 0℃ in an ice bath. 64 µl of Tf_2O (385 µmol, 3 equiv.) were added and the solution was stirred under Ar for 10 min. 0.10 g of **56** (0.13 mmol, having two equiv reacting centers in the molecule) were added and the solution was further stirred under Ar, letting it warm up to rt. The reaction was monitored on TLC (H:AcOEt 1:10). The solution was then diluted with CH_2Cl_2, washed with dilute $NaHCO_3$, the aq. layer back extracted with CH_2Cl_2, the comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. The crude material were dissolved in CH_2Cl_2, adsorbed on SiO_2 and chromatographically purified on SiO_2 with H:AcOEt 1:5. 51 mg (74 µmol, 58%) of the colourless solid **81** were isolated.

R_f(H/AcOEt 1:10): 0.2



81

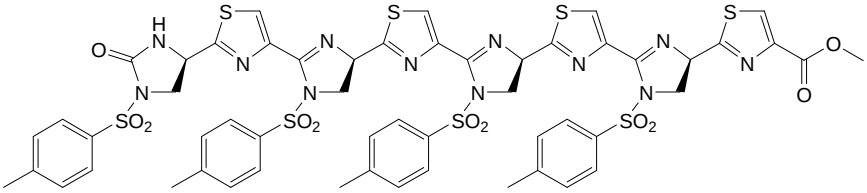
^{1}H-NMR (300 MHz, DMSO): 8.71 (br. *s*, 1 H, NH); 8.26 (*t*, ^3J = 6.4, 1 H, NCH); 8.11; 7.87 (2 *s*, 2 H, Thiazole-CH); 7.82 (*d*, ^3J = 8.1, 2 H, Ts-CH); 7.63 (*d*, ^3J = 8.0, 2 H, Ts-CH); 7.40 (*d*, ^3J = 8.2, 2 H, Ts-CH); 7.30 (2 *d*, ^3J = 8.3, 2 H, Ts-CH); 5.10 (*dd*, ^2J = 4.1, ^3J = 9.1, 1 H); 4.31 (*t*, ^3J = 9.1, 1 H); 4.07 (*d*, ^3J = 6.3, 2 H, CH_2); 3.91 (*dd*, ^3J = 4.1, ^2J = 9.4, 1 H); 3.62 (*s*, 3 H, OCH_3); 2.40; 2.33 (2 *s*, 6 H, Ts-CH_3).

MS (ESI, $CHCl_3$/MeOH 3:1 + Na): 709.2 (100, $[M + Na]^+$); 301.1 (74, $[M_{Ph3PO} + Na]^+$).

b. Synthesis of **88**

In a dry 5 ml flask 141 mg of Ph_3PO (507 µmol, 12 equiv.) were dissolved in 1 ml dry CH_2Cl_2 (*puriss. p.a.*) under N_2 and the solution was cooled down to 0℃. 42 µl of Tf_2O (255 µmol, 6 equiv.) were added. After 10 min a precipitate has formed. 60 mg of **53** (42 µmol) were added and after 5 min all substances have dissolved again. The solution was let warm up to rt while stirring. Reaction was monitored on TLC (H:AcOEt 1:10). After 2.5 h the solution was diluted with AcOEt, washed with 0.1 M citric acid and once with brine. All aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and the solvent removed in rv. The crude

material was further purified chromatographically on SiO_2 (H:AcOEt 1:10 to pure AcOEt). 17 mg of the not completely pure product **88** (around 13 µmol, 31%) were isolated.



88

R_f(H/AcOEt 1:10): 0.15

MS (ESI, MeOH/DMSO 9:1 + NaI): 1337.2 (30, [*M* + H_2O + Na]$^+$); 1319.2 (100, [*M* + Na]$^+$); 1303.2 (20).

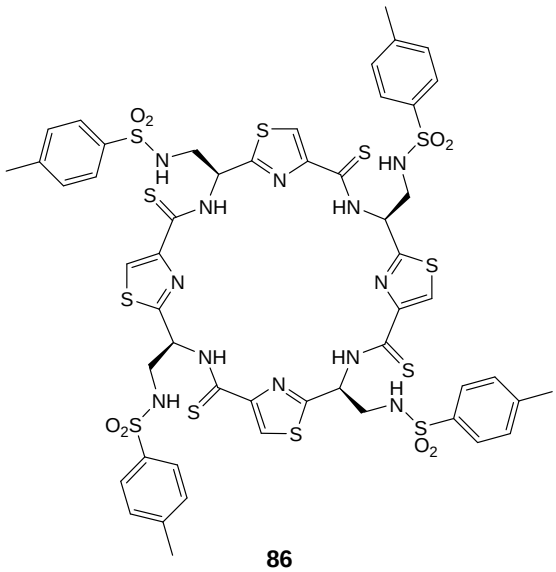
8.1.5.2 Reactions on macrocycle **58**

8.1.5.2.1 Synthesis of tetrathioamide macrocycle **86**

90 mg of **58** (70 µmol) were dissolved in 12 ml dry pyridine (*puriss. p.a.*) and 309 mg of P_2S_5 (1.39 mmol, 4 x 5 equiv.) were added. The suspension was heated up to reflux (oil bath 150°C) for 5 h under N_2. The progress of the reaction was monitored on TLC ($CHCl_3$:MeOH 6:1). The solvent was then removed under reduced pressure freezing it out in a cooling trap, the remaining material was suspended in a mixture of $CHCl_3$ and MeOH, the solid component was filtered off, the dissolved one adsorbed at SiO_2. Filtration over SiO_2 (CH_2Cl_2:MeOH 25:1) yielded 24 mg of the quite pure (3*S*,11*S*,19*S*,27*S*) 3,11,19,27-tetra[(tosylamino)methyl]-1,9,17,25-tetrathio-2,10,18,26-tetraza[3.$_4$](2,4)1,3-thiazolophan (**86**) (18 µmol, 26%).

R_f(H/AcOEt 1:3): 0.6

MS (ESI, MeOH + NaI): 1529.0 (5); 1379.1 (100, [*M* + Na]$^+$); 1196.1 (11).



86

8.2 Target 2: [0.$_8$](2,4)1,3-Thiazolophan (34)

8.2.1 Synthesis of Boc-TSer(OBn)-OMe (*rac*-**89**)

8.2.1.1 Synthesis of **39**

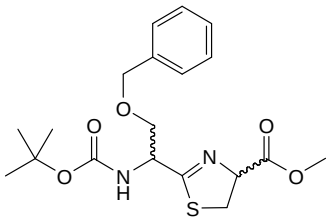
See section 8.1.1.2.

8.2.1.2 Synthesis of **91**

2.93 g of **39** (9.94 mmol) were dissolved in ca. 100 ml CH_2Cl_2 (*purum*) under Ar. 3.01 g of $CaCO_3$ (30.1 mmol, 3 equiv.), 2.10 g of Et_3OBF_4 (11.0 mmol, 1.1 equiv.) were added and the suspension was stirred at rt. The reaction was monitored by TLC (H:AcOEt 1:1). After 9 h the solution containing intermediate **40** was filtered over 2 cm SiO_2 and diluted with CH_2Cl_2 to 200 ml.

1.89 g of cysteine hydrochloride methyl ester (11.0 mmol, 1.1 equiv.) were suspended in ca. 100 ml CH_2Cl_2 (*puriss. p.a.*) and 1.4 ml of Et_3N (10 mmol, 1 equiv.) were added. The 200 ml solution containing **40** was added during 10 min and the suspension was stirred at rt overnight. The progress of the reaction was monitored on TLC (H:AcOEt 1:1). Suspension was then filtered to remove the precipitated NH_4Cl and the solvent was removed in rv. The remaining oil was dissolved in TBME, washed twice with 0.1 M citric acid and once with brine. All aq. layers were back extracted with TBME and the comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. 3.20 g of the crude product **91** were isolated and chromatographically purified over SiO_2 (H:AcOEt 4:1 to 2:1). 2.54 g (6.44 mmol, 68%) of the pure methyl 2-{2-(benzyloxy)-1-[(*tert*-butoxycarbonyl)amino]ethyl}-thiazoline-4-carboxylate (**91**) were isolated as colourless oil.

R_f(Hexane/AcOEt 2:1): 0.26
IR (film on NaCl): 3360*m*; 3063*w*; 3030*w*; 2977*m*; 2952*m*; 2932*m*; 2867*m*; 1742*s*; 1716*s*; 1618*m*; 1496*s*; 1454*s*; 1437*m*; 1392*m*; 1366*s*; 1247*s*; 1201*s*; 1168*s*; 1104*s*; 1047*m*; 1026*s*; 933*w*; 864*w*; 779*w*; 739*m*; 698*m*; 604*w*.



91

^{1}H-NMR (300 MHz, $CDCl_3$): 7.34 – 7.26 (*m*, 5 H, arom. CH); 5.52 (*d*, ^3J = 6.8, 1 H, NHC); 5.11 (*dd*, $^3J_{cis}$ = 9.5, $^3J_{trans}$ = 1.8, 1 H, NCH); 4.72 (br. *s*, 1 H, Thiazoline-CH); 4.54 (*s*, 2 H, CH_2Ph); 3.85 (br. *s*, 1 H, *trans*-H, CH_2); 3.79 (*s*, 3 H, OMe); 3.75 (*dd*, $^3J_{cis}$ = 9.5, ^2J = 4.3, 1 H, *cis*-H, CH_2); 3.63 – 3.47 (*m*, 2 H, Thiazoline-CH_2); 1.45 (*s*, 9 H, *t*-Bu).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 176.41 (*s*, 1 C, Thiazoline-CNS); 171.03 (*s*, 1 C, COOMe); 155.14 (*s*, 1 C, Carbamat-CO); 137.69 (*s*, 1 C, arom. C); 128.39; 127.76; 127.61 (3 *d*, 5 C, arom. CH); 80.08 (*s*, 1 C, $OCMe_3$); 78.30 (*d*, 1 C, Thiazoline-CH); 73.28; 70.98 (2 *t*, 2 C, 2 OCH_2); 53.46 (*d*, 1 C, NCH); 52.77 (*q*, 1 C, OCH_3); 35.23 (*t*, 1 C, Thiazoline-CH_2); 28.32 (*q*, 3 C, $C(CH_3)_3$).

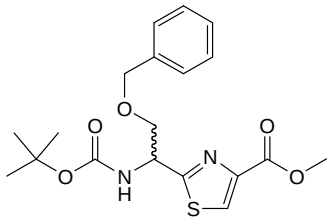
MS (ESI, MeOH/$CHCl_3$ 1:1 + NaI): 433.1 (4, $[M + K]^+$); 417.2 (50, $[M + Na]^+$); 395.2 (100, $[M + H]^+$); 339.2 (20, $[M – CMe_3 + 2 H]^+$); 287.1 (5, $[M$ – O-Bn + 2 H$]^+$); 231.0 (4, $[M – CMe_3$ – O-Bn + 3 H$]^+$).

8.2.1.3 Synthesis of Boc-TSer(OBn)-OMe (**89**)

2.42 g of **91** (6.13 mmol) were dissolved in 100 ml CH_2Cl_2 (*purum*) and cooled down to 0℃. 1.86 ml of DBU (12.3 mmol, 2 equiv.) and 0.63 ml of $BrCCl_3$ (6.39 mmol, 1.05 equiv.) were added[182, 203] and the solution was stirred for 30 min at 0℃, then ice bath was removed. The reaction was monitored by TLC (H:AcOEt 2:1). After one hour the solvent was removed, the remaining oil dissolved in AcOEt, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, the comb. org. layers dried over $MgSO_4$, filtered and dried in rv. 2.64 g of the crude **89** were purified over SiO_2 (H:AcOEt 5:1 to 3:1). 2. 36 g (6.03 mmol, 98%) of the pure methyl 2-{(*rac*)-2-(benzyloxy)-1-[(*tert*-butoxycarbonyl)amino]ethyl}-1,3-thiazole-4-carboxylate (Boc-TSer(OBn)-OMe, **89**) were isolated.

R_f(H/AcOEt 2:1): 0.2
IR (KBr): 3451*m*; 3238*s*; 3127*m*; 3061*m*; 2976*m*; 2927*m*; 2855*m*; 1741*s*; 1696*s*; 1553*s*; 1498*m*; 1479*m*; 1453*m*; 1430*m*; 1415*m*; 1386*m*; 1364*s*; 1346*m*; 1296*s*; 1274*m*; 1216*s*; 1171*s*; 1121*s*; 1101*s*; 1070*m*; 1048*m*; 1023*m*; 994*m*; 979*m*; 915*w*; 903*w*; 891*w*; 869*m*; 831*w*; 820*w*; 809*w*; 785*m*; 761*m*; 735*s*; 713*w*; 697*m*; 666*w*; 653*w*; 641*w*; 630*w*; 615*m*.

^{1}H-NMR (300 MHz, $CDCl_3$): 8.12 (s, 1 H, Thiazole-H); 7.34 – 7.19 (*m*, 5 H, arom. CH); 5.67 (br. *s*, 1 H, NH)); 5.20 (br. *s*, 1 H, NCH); 4.52; 4.47 (2 *d*, ^2J = 11.9, 2 H, OCH_2Ph); 4.02 (br. *s*, 1 H, CH_2); 3.94 (*s*, 3 H, OCH_3); 3.86 (*dd*, ^2J = 9.5, ^3J = 4.5, 1 H, CH_2); 1.45 (*s*, 9 H, $C(CH_3)_3$).



89

^{13}C-NMR (75.5 MHz, $CDCl_3$): 172.89 (*s*, 1 C, Thiazole NCS); 161.78 (*s*, 1 C, COOMe); 155.15 (*s*, 1 C, Carbamat-CO); 146.82 (*s*, 1 C, Thiazole-C); 137.34 (*s*, 1 C, arom. C); 128.38; 127.80; 127.69; 127.58 (*d*, 6 C, 5 arom. CH, Thiazole-CH); 80.38 (*s*, $OCMe_3$); 73.32; 71.39 (2 *t*, 2 C, CH_2OCH_2); 53.16 (*d*, 1 C, CH); 52.34 (*q*, 1 C, OCH_3); 28.23 (*q*, 3 C, $OC(CH_3)_3$).

MS (ESI, MeOH): 431.2 (5, $[M + K]^+$); 415.3 (100, $[M + Na]^+$); 359.2 (7, $[M – C(CH_3)_3 + 2\ H]^+$); 337.2 (14, $[M – C(CH_3)_3 + H + Na]^+$); 293.2 (9, $[M – COOC(CH_3)_3 + 2\ H]^+$).

X-ray crystallography: A nice crystal of **89**, sufficient for x-ray analysis, could be obtained by slow evaporation of a mixture of CH_2Cl_2 and AcOEt.

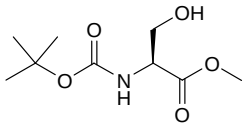
8.2.2 Synthesis of L-Boc-TSer(OAll)-OMe (**90**)

8.2.2.1 Synthesis of **46**

10.01 g of H-Ser-OMe.HCl (64.3 mmol) were suspended in ~150 ml freshly distilled CH_2Cl_2 in a fire dried flask under N_2 and stirred mechanically. 20 ml of Et_3N (0.14 mol, 2.2 equiv) were added and the suspension was cooled down to 0℃ in an ice bath. After around 30 min 15.50 g Boc_2O (71.02 mmol, 1.1 equiv.) were added and the suspension was further stirred for at least 3 h or overnight[237]. The reaction was monitored by TLC (H:AcOEt 2:1) staining with ninhydrin. The suspension was then diluted with CH_2Cl_2, washed three times with 1 M $NaHSO_4$, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with CH_2Cl_2 and the comb.

org. layers dried over $MgSO_4$, filtered and solvent removed in rv. 14.31 g (65.3 mmol, 101%) pure L-Boc-Ser-OMe (**46**) were isolated.

R_f(H/AcOEt 3:1): 0.06



46

^{1}H-NMR (300 MHz, $CDCl_3$): 5.49 (br. *s*, 1 H, NH); 4.40 (br. *s*, 1 H, CH); 3.94 (*dd*, ^2J = 10.1, ^3J = 3.7, 2 H, CH_2); 3.79 (*s*, 3 H, OCH_3); 1.45 (*s*, 9 H, $C(CH_3)_3$).

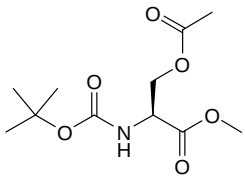
^{13}C-NMR (75.5 MHz, $CDCl_3$): 171.20 (*s*, 1 C, *C*$OOCH_3$); 155.65 (*s*, 1 C, COON); 80.26 (*s*, 1 C, $OCMe_3$), 63.47 (*t*, 1 C, CH_2), 55.60 (*d*, 1 C, CH); 52.56 (*q*, 1 C, OCH_3); 28.18 (*q*, 3 C, $C(CH_3)_3$).

MS (MeOH + NaI): 461.0 (5); 342.1 (17, $[M - H + COOCMe_3 + Na]^+$); 298.1 (7); 286.0 (15, $[M + COOCMe_3 - CMe_3 + Na]^+$); 242.0 (100, $[M + Na]^+$); 185.9 (26, $[M - CMe_3 + H + Na]^+$).

8.2.2.2 Synthesis of **47**

14.87 g of **46** (67.9 mmol) were dissolved in ~150 ml CH_2Cl_2 (*purum*) and cooled down to 0°C in an ice bath. 18.9 ml of Et_3N (136 mmol, 2 equiv.), 250 mg of DMAP (2.05 mmol, 3 mol%) and 9.6 ml of Ac_2O (102 mmol, 1.5 equiv.) were added. The colourless solution was stirred under N_2. After 15 min ice bath was removed and the solution was warmed up to rt. The reaction was monitored on TLC (H:AcOEt 2:1, staining with ninhydrin). After 90 min reaction was finished. The solution was diluted with CH_2Cl_2, washed once with 1 M $NaHSO_4$, once with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aqueous layers were back extracted with CH_2Cl_2, the comb. org. layers were dried over $MgSO_4$, filtered and the solvent removed in rv. The 16.61 g of the slightly yellow oil **47** were distilled in “Kugelrohr oven” (first fraction (impurity): 15 mbar, 140-150°C, second (product): 15 mbar, ~160°C) and 14.89 g (56.97 mmol, 84%) of the pure L-Boc-Ser(OAc)-OMe (**47**) were collected as a colourless oil.

R_f(H/AcOEt 2:1): 0.30; R_f(H/AcOEt 3:1): 0.17



47

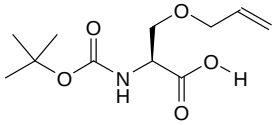
^{1}H-NMR (300 MHz, $CDCl_3$): 5.30 (br. *d*, *J* = 4.6, 1 H, NH); 4.59 – 4.56 (*m*, 1 H, CH); 4.44 (br. *dd*, ^2J = 11.1, ^3J = 3.7, 1 H, CH_2); 4.33 (*dd*, ^2J = 11.1, ^3J = 3.6, 1 H, CH_2); 3.77 (*s*, 3 H, $(CO)OCH_3$); 2.06 (*s*, 3 H, $(CO)CH_3$); 1.46 (*s*, 9 H, $C(CH_3)_3$).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 170.49; 170.30 (2 *s*, 2 C, 2 COO); 155.18 (*s*, 1 C, O(CO)NH); 80.36 (*s*, 1 C, $OCMe_3$); 64.27 (*t*, 1 C, CH_2O); 52.94 (*d*, 1 C, CH); 52.73 (*q*, 1 C, $(CO)OCH_3$); 28.30 (*q*, 1 C, $OC(CH_3)_3$); 20.67 (*q*, 1 C, $(CO)CH_3$).

MS (ESI, MeOH+ NaI): 284.1 (100, $[M + Na]^+$), 228.0 (27, $[M - C(CH_3)_3 + H + Na]^+$).

8.2.2.3 Synthesis of **92**

8.50 g of Boc-Ser-OH (41.4 mmol) were dissolved in 60 ml dry DMF (solvent system). In another flask 4.14 g of the 60% NaH on paraffin (104 mmol, 2.5 equiv.) were mixed with 30 ml dry DMF (solvent system) and cooled down to 0°C. The solution of Boc-Ser-OH was slowly added through a dropping funnel. A vigorous evolution of H_2 was observed. After complete addition, 3.5 ml of allylbromide (41 mmol, 1 equiv.) were added. The solution was slowly let warm up to rt and stirred overnight[238]. The remaining NaH was then quenched by addition of some H_2O, a part of the solvent was removed by rv, the remaining oily substance diluted with H_2O and washed twice with hexane. The org. layers were back extracted with little 1 M NaOH, the comb. aq. layers acidified with 6 M HCl to pH 3 – 4 and extracted three times with AcOEt. The comb. AcOEt layers were washed once with 0.1 M aq. citric acid and once with 0.1 M citric acid in brine, dried over $MgSO_4$, filtered and the solvent removed in rv. 9.44 g pure L-Boc-Ser(OAll)-OH (**92**) (38.5 mmol, 93%) were isolated.



92

^{1}H-NMR (300 MHz, $CDCl_3$): 5.93 – 5.80 (*m*, 1 H, Allyl-CH); 5.40 (br. *d*, ^3J = 8.4, 1 H, NH); 5.30 – 5.19 (*m*, 2 H, Allyl-C=CH_2); 4.46 – 4.44 (*m*, 1 H, NCH); 4.02 (*dt*, ^3J = 5.3, ^4J = 1.0, 2 H, Allyl-OCH_2); 3.91 (b. *dd*, ^2J = 9.5, ^3J = 3.4, 1 H, OCH_2); 3.67 (*dd*, ^2J =9.5, ^3J = 3.9, 1 H, OCH_2); 1.46 (*s*, 9 H, $OC(CH_3)_3$).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 174.04 (*s*, 1 C, COOH); 155.72 (*s*, 1 C, Carbamat-CO); 134.09 (*d*, 1 C, Allyl-CH); 117.50 (*t*, 1 C, Allyl-C=CH_2); 80.11 (*s*, 1 C, $OCMe_3$); 72.35; 69.80 (2 *t*, 2 C, CH_2OCH_2); 53.86 (*d*, 1 C, NCH); 28.33 (*q*, 3 C, $OC(CH_3)_3$).

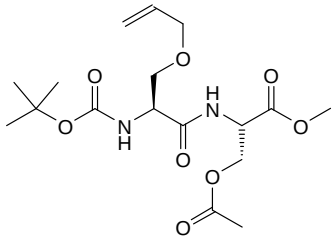
8.2.2.4 Synthesis of **50**

0.52 g of **47** (2.0 mmol) were dissolved in 5 ml dry CH_2Cl_2 (freshly distilled over CaH_2). A mixture of 2.5 ml CH_2Cl_2 and 2.5 ml TFA was added and the solution was stirred at rt for 3 h under N_2. Reaction was monitored on TLC (H:AcOEt 2:1, staining with ninhydrin). Solvent was then removed by rv, freezing it out in a cooling trap. The oily residue was dissolved in MeOH, mixed with Amberlyst in its Cl^--form during 2 h, filtered and dried again in rv and hv. Amine **50** was directly used for peptide coupling.

8.2.2.5 Synthesis of **93**

2.40 g of **92** (9.77 mmol) were dissolved in 100 ml CH_2Cl_2. 2.2 ml of *N*-methylmorpholine (19.5 mmol, 2 equiv.) and 3.12 g of TBTU (9.72 mmol, 1 equiv.) were added. 1 equiv. **50** (9.72 mmol, prepared as described above), dissolved in 100 ml CH_2Cl_2 and 2.2 ml of NMM (19.5 mmol, 2 equiv.) were added. The mixture was stirred at rt under N_2 for 5 h. The reaction was monitored on TLC (H:AcOEt 2:1). A part of the solvent was removed, the rest diluted with TBME, washed three times with aq. 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME, the comb. org. layers dried over $MgSO_4$, filtered and dried in rv. The isolated 4.11 g crude **93** were chromatographically purified on SiO_2 (hexane:AcOEt 2:1). 2.55 g pure Boc-Ser(OAll)-Ser(OAc)-OMe **93** (6.58 mmol, 67%) were isolated.

R_f(H/AcOEt 2:1): 0.35


93

^{1}H-NMR (300 MHz, $CDCl_3$): 7.34 (br. *s*, 1 H, Amide-NH); 5.96 – 5.83 (*m*, 1 H, Allyl-CH), 5.42 (br. *s*, 1 H, Carbamat-NH); 5.32 – 5.18 (*m*, 2 H, Allyl-C=CH_2); 4.86 – 4.81 (*m*, 1 H, *N*-terminal NCH); 4.46 (*dd*, ^2J = 11.1, ^3J = 3.8, 1 H, $AcOCH_2$); 4.35 (*dd*, ^2J = 11.4, ^3J = 3.4, 1 H, $AcOCH_2$); 4.29 (br. *s*, 1 H, *C*-terminal NCH); 4.05 (br. *d*, ^3J = 5.3, 2 H, Allyl-OCH_2); 3.86 (*dd*, ^2J = 9.2, ^3J = 3.8, 1 H, CH_2OAll); 3.78 (*s*, 3 H, OCH_3); 3.54 (*dd*, ^2J = 9.2, ^3J= 7.4, 1 H, CH_2OAll); 2.05 (*s*, 3 H, $(CO)CH_3$); 1.47 (*s*, 9 H, $OC(CH_3)_3$).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 170.42; 170.26; 169.52 (3 *s*, 3 C, 3 COO); 155.92 (*s*, 1 C, Carbamat-CO); 133.91 (*d*, 1 C, Allyl-CH); 117.46; 117.42 (2 *t*, 1 C, Allyl-CH_2); 80.25 (*s*, 1 C, $OCMe_3$); 72.21; 72.16; 69.53; 69.32; 63.67; 63.54 (6 *t*, 3 C, 3 OCH_2); 53.67; 52.80 (2 *d*, 2 C, 2 NCH); 51.71 (*q*, 1 C, OCH_3); 28.23 (*q*, 3 C, $OC(CH_3)_3$); 20.60 (*q*, 1 C, $(CO)CH_3$).
The occurrence of doubled signals indicates the existence of diastereoisomers. Apparently at least one of the steregenic centers has racemised during the synthesis.

MS (ESI, MeOH/$CHCl_3$ 1:1 + NaI): 451.2 (5); 411.2 (100, $[M + Na]^+$).

8.2.2.6 Synthesis of **94a** and **94b** (mixture)

2.54 g of **93** (6.55 mmol) were dissolved in 25 ml toluene (*purum*) and heated to 125℃ (reflux) under N_2. 1.32 g of Lawesson reagent (3.27 mmol, 0.5 equiv.) were added and the solution was stirred for two hours. Reaction was monitored on TLC (H:AcOEt 2:1)[4]. The solution was diluted with TBME, washed twice with 0.1 M aq. citric acid, twice with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME, the comb. org. layers were dried over $MgSO_4$, filtered and solvent was removed in rv. 3.33 g of the crude product was isolated as a yellowish, smelling oil. The oily substance was purified chromatographically on SiO_2 (H:AcOEt 3:1). 2.61 g of Boc-ThiaSer(OAll)-Ser(OAc)-OMe **94a** (6.44 mmol, 98%) could be isolated.

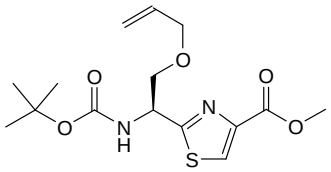
8.2.2.7 Synthesis of L-Boc-TSer(OAll)-OMe (**90**)

2.61 g of **94** (6.44 mmol) were dissolved in 100 ml CH_2Cl_2, 3.4 ml of DBU (23 mmol, 3.5 equiv.) and 0.76 ml of $BrCCl_3$ (7.7 mmol, 1.2 equiv.) were added and the solution was stirred at rt under N_2 for 3 h. The reaction was monitored on TLC (H:AcOEt 2:1). A part of the solution was removed by rv, the remaining brown oil diluted with TBME, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME, the comb. org. layers were dried over Na_2SO_4, filtered and solvent was removed in rv. 1.90 g (5.54 mmol, 86%) of the crude product were isolated and chromatographically purified on SiO_2 (H:AcOEt 4:1). 1.61 g (4.70 mmol, 73%) of the pure methyl 2-{2*S*-2-(benzyloxy)-1-[(*tert*-butoxycarbonyl)amino]ethyl}-thiazole-4-carboxylate (L-Boc-TSer(OAll)-OMe, **90**) could be isolated. Oil **90** solidified after some days.

R_f(H/AcOEt 2:1): 0.45

[4] longer reaction time favours the evolution of the in principle desired oxazolidine **94b**, the following oxidation reaction does not depend on nature of **94** and therefore normally mixtures of **94a** and **94b** were isolated and oxidized as such

^{1}H-NMR (300 MHz, $CDCl_3$): 8.12 (*s*, 1 H, Thiazole-CH); 5.88 – 5.75 (*m*, 1 H, Allyl-CH); 5.68 (br. *s*, 1 H, NH); 5.24 – 5.13 (*m*, 3 H, Allyl-C=CH_2, NCH); 4.00 – 3.95 (*m*, 3 H, Allyl-O-CH_2, 1xOCH_2); 3.94 (*s*, 3 H, OCH_3); 3.81 (*dd*, ^2J = 9.5, ^3J = 4.5, 1 H, OCH_2); 1.46 (*s*, 9 H, $OC(CH_3)_3$).



90

^{13}C-NMR (75.5 MHz, $CDCl_3$): 172.94 (*s*, 1 C, Thiazole-CNS); 161.86 (*s*, 1 C, COOMe); 155.16 (*s*, 1 C, Carbamat-CO); 146.85 (*s*, 1 C, Thiazole-C); 133.90 (*d*, 1 C, Allyl-CH); 127.78 (*d*, 1 C, Thiazole-CH); 117.50 (*t*, 1 C, Allyl-C=CH_2); 80.45 (*s*, 1 C, $OCMe_3$)); 72.25; 71.35 (2 *t*, 2 C, CH_2OCH_2); 53.14 (*d*, 1 C, NCH); 52.43 (*q*, 1 C, OCH_3); 28.30 (*q*, 3 C, $OC(CH_3)_3$).

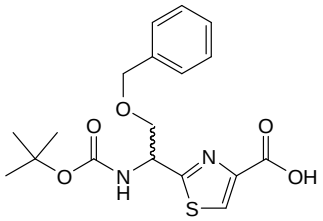
MS (ESI, MeOH/$CHCl_3$ 1:1 + NaI): 365.2 (100, $[M + Na]^+$); 309.1 (6, $[M – C(CH_3)_3 + H + Na]^+$).

8.2.3 Synthesis of tetramer Boc-[TSer(OBn)]$_4$-OMe (**95**)

8.2.3.1 Synthesis of **96**

9.98 g of **89** (25.5 mmol) were dissolved in 100 ml MeOH at rt under N_2. 3.67 g of LiOH (153 mmol, 6 equiv.) were dissolved in 50 ml H_2O and added to the solution of **89**. The reaction was monitored on TLC (H:AcOEt 2:1). After completion a part of the solvent was removed in rv. The remaining solution was acidified with 1 M $NaHSO_4$, whereas a colourless precipitate formed which was twice extracted with AcOEt. The org. layers were washed with 0.1 M citric acid in brine, then combined, dried over $MgSO_4$, filtered and dried in rv and hv. The isolated acid **96** was directly used for peptide bond formation.

^{1}H-NMR (300 MHz, $CDCl_3$): 9.80 (br. *s*, 1 H, OH); 8.23 (*s*, 1 H, Thiazole-H); 7.34 – 7.21 (*m*, 5 H, arom. CH); 5.76 (br. *d*, 1 H, NH); 5.24 (br. *s*, 1 H, NCH); 4.54; 4.49 (2 *d*, ^2J = 12.2, 2 H, OCH_2Ph); 4.04 (br. *s*, 1 H, CH_2); 3.86 (*dd*, ^2J = 9.1, ^3J = 4.1, 1 H, CH_2); 1.46 (*s*, 9 H, $OC(CH_3)_3$).



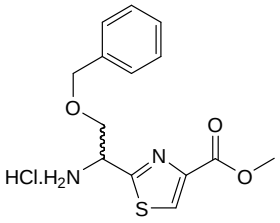
96

^{13}C-NMR (75.5 MHz, $CDCl_3$): 172.98 (*s*, 1 C, Thiazole NCS); 164.14 (*s*, 1 C, COOH); 155.27 (*s*, 1 C, Carbamat-CO); 146.35 (*s*, 1 C, Thiazole-C); 137.29 (*s*, 1 C, arom. C); 128.95; 127.46; 127.92; 127.69 (*d*, 6 C, Thiazole-CH, 5 arom. CH); 80.61 (*s*, $OCMe_3$); 73.40; 71.28 (2 *t*, 2 C, CH_2OCH_2); 53.06 (*d*, 1 C, CH); 28.28 (*q*, 3 C, $OC(CH_3)_3$).

MS (ESI, MeOH): 401.3 (100, $[M + Na]^+$); 345.2 (15, $[M – CMe_3 + H + Na]^+$); 323.2 (31, $[M – CMe_3 + 2 H]^+$); 320.2 (8); 301.3 (5, $[M – CO_2CMe_3 + H + Na]^+$); 279.2 (20, $[M – CO_2CMe_3 + 2 H]^+$); 150.1 (14).

8.2.3.2 Synthesis of **97**

9.96 g of **89** (25.5 mmol) were dissolved in 45 ml CH_2Cl_2 at rt under N_2 and 15 ml TFA were added. The reaction was monitored on TLC (H:AcOEt 2:1). The solvent was then removed under reduced pressure freezing it out in a cooling trap and the remaining oil dried in hv, then dissolved again in MeOH and stirred at rt under N_2 for 16 h over Amberlyst-A26 in it's Cl^--form. The solid was then removed by filtration and the solvent in rv and hv. The isolated **97** was directly used for peptide bond formation.



97

MS (ESI, CH_2Cl_2/MeCN 1:3 + NaI): 315.1 (10, $[M + Na]^+$); 293.2 (100, $[M + H]^+$).

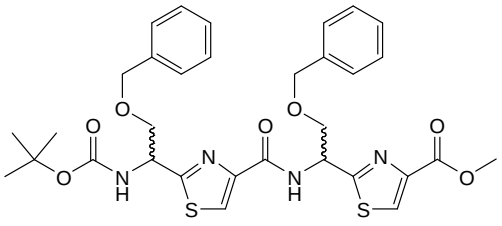
8.2.3.3 Synthesis of Boc-[TSer(OBn)]$_2$-OMe (**98**)

25.45 mmol of **96** (directly form deprotection) were dissolved in 150 ml CH_2Cl_2 and 7.0 ml of NMM (62 mmol, 2.5 equiv.).

25.45 mmol of **97** (directly from deprotection) were dissolved in 150 ml CH_2Cl_2 and 7.0 ml of NMM (62 mmol, 2.5 equiv.).

8.17 g of TBTU (25.4 mmol, 1 equiv.) and the solution of **97** were added to the solution of **96**. The mixture was stirred at rt under N_2. The reaction was monitored on TLC (H:AcOEt 1:1). After 16 h a part of the solvent was removed by rv, the remaining oil diluted with AcOEt, washed with 1 M $NaHSO_4$, 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with AcOEt, the comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv and hv. 16.83 g of the crude product were obtained and chromatographically purified on SiO_2 (H:AcOEt 2:1, to 3:2 and 1:1). 11.60 g Boc-[TSer(OBn)]$_2$-OMe **98** (17.79 mmol, 70%) were isolated.

R_f(H/AcOEt 1:1): 0.3
$[\alpha]_D^{20}$ ($CHCl_3$): + 2.1
IR (KBr): 3408*s*; 3114*m*; 3030*m*; 2927*m*; 2865*m*; 1717*s*; 1670*s*; 1536*s*; 1496*s*; 1454*s*; 1384*m*; 1366*s*; 1324*m*; 1264*s*; 1214*s*; 1167*s*; 1100*s*; 1022*s*; 913*w*; 862*w*; 826*w*; 805*w*; 752*s*; 698*s*; 607*m*; 518*w*; 499*w*; 489*w*; 479*w*; 469*w*; 461*w*.



98

^{1}H-NMR (400 MHz, DMSO): Mixture of diastereoisomers: 9.19 – 8.89 (*m*, 1 H, Amid-NH); 8.79; 8.51; 8.49; 8.48; 8.32 (5 *s*, 2 H, Thiazole-CH); 7.86 – 7.84 (*m*, 1 H, Carbamat-NH); 7.34 – 7.26 (*m*, 10 H, arom. CH); 5.69 – 5.70 (*m*, 1 H, NCH); 5.16 – 5.14 (*m*, 1 H, NCH); 4.61 – 4.52 (*m*, 4 H, OCH_2Ph); 4.10 – 3.95 (*m*, 3 H, 2 CH_2); 3.84 (*s*, 3 H, OCH_3); 3.84 – 3.81 (*m*, 1 H, CH_2); 1.42 (*s*, 9 H, $OC(CH_3)_3$).

^{1}H-NMR (300 MHz, $CDCl_3$): Mixture of diastereoisomers: 8.25 (br. *d*, ^3J = 8.1, Amide-NH); 8.12; 8.11; 8.09 (3 *s*, 2 H, Thiazole-CH); 7.33 – 7.19 (*m*, 10 H, arom. CH); 5.72 – 5.66 (*m*, 2 H, 2 NCH); 5.18 (br. *s*, 1 H, Carbamate-NH); 4.56 – 4.45 (*m*, 4 H, 2 OCH_2Ph); 4.18 – 4.10 (*m*, 1 H, CH_2); 3.99 – 3.95 (*m*, 2 H, CH_2); 3.93; 3.93 (2 *s*, 3 H, OCH_3); 3.88 (br. *dd*, ^2J = 9.4, ^3J = 4.3, CH_2); 1.46 (*s*, 9 H, $OC(CH_3)_3$).

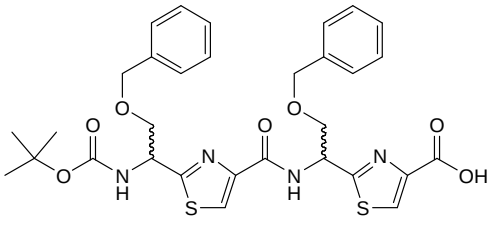
^{13}C-NMR (75.5 MHz, $CDCl_3$): 172.15; 171.16 (2 *s*, 2 C, 2 Thiazole-CNS); 161.76; 160.95 (2 *s*, 2 C, COOMe, CONH); 155.21 (*s*, 1 C, Carbamate-CO); 149.02; 146.85 (2 *s*, 2 C, Thiazole-C); 137.44; 137.37 (2 *s*, 2 C, 2 arom. C); 128.46; 128.42; 127.89; 127.84; 127.81; 127.67; 127.62; 127.58 (8 *d*, 12 C, 10 arom. CH, 2 Thiazole CH); 80.58 (*s*, 1 C, $OCMe_3$); 73.46; 73.41; 73.35; 73.31; 71.34; 70.90 (6 *t*, 4 C, 2 CH_2OCH_2); 53.09; 52.40 (2 *d*, 2 C, 2 NCH); 51.54 (*q*, 1 C, OCH_3); 28.32 (*q*, 3 C, $OC(CH_3)_3$).

MS (ESI, MeOH): 691.3 (24, $[M + K]^+$); 675.4 (100, $[M + Na]^+$); 653.4 (5, $[M + H]^+$); 597.3 (11, $[M + - CMe_3 + 2 H]^+$); 553.3 (8, $[M + - CO_2CMe_3 + 2 H]^+$).
MS (ESI, CH_2Cl_2/MeCN 1:3 + NaI): 675.4 (100, $[M + Na]^+$).

8.2.3.4 Synthesis of **146**

0.42 g **98** (0.64 mmol) were dissolved in 20 ml MeOH and 89 mg of LiOH (3.7 mmol, 6 equiv.), dissolved in 10 ml H_2O, were added. The reaction was monitored on TLC (H:AcOEt 1:1). After 6 h a part of the solvent was removed by rv. The remaining solution was acidified with 0.1 M citric acid, whereas a colourless precipitate formed which was twice extracted with AcOEt. The org. layers were washed with 0.1 M citric acid in brine, combined, dried over $MgSO_4$, filtered and solvent removed in rv and hv. The so isolated **146** was directly used for peptide bond formation.

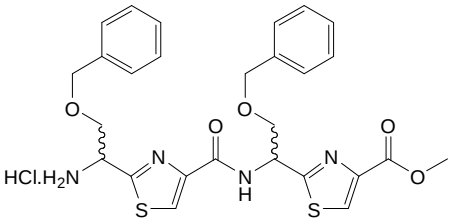
8.2.3.5 Synthesis of **147**



146

0.40 g **98** (0.61 mmol) were dissolved in 15 ml CH_2Cl_2 under N_2 and 15 ml TFA were added. Reaction was monitored on TLC (H:AcOEt 1:1). The solvent was then removed under reduced pressure freezing it out in a cooling trap and the remaining oil dried in hv. It was then dissolved in MeOH and stirred at rt under N_2 for 16 h over Amberlyst-A26 in it's Cl^--form, then filtered, dried in rv and hv. The obtained **147** was directly used for peptide bond formation.

8.2.3.6 Synthesis of Boc-[TSer(OBn)]$_4$-OMe (**95**)



147

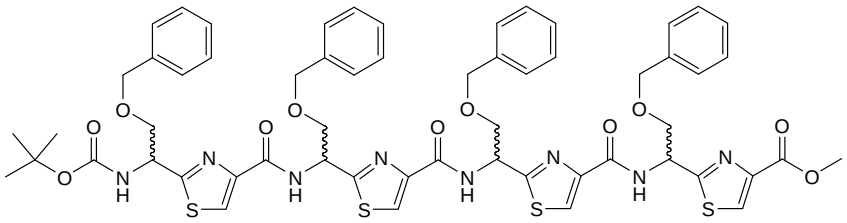
0.64 mmol of **146** (1 equiv.), directly after deprotection as described above, were dissolved in 25 ml DMF and 200 µl of NMM (1.82 mmol, 3 equiv.) were added. pH was tested to be basic.

0.607 mmol of **147** (1 equiv.), directly after deprotection as described above, were dissolved in 25 ml DMF and 200 µl of NMM (1.82 mmol, 3 equiv.) were added. pH was tested to be basic.

470 mg of PyBOP (903 µmol, 1.5 equiv.) and the solution of **147** were added to the solution of **146**. The suspension was stirred at rt under N_2. The formation of product **95** was monitored on TLC (H:AcOEt 1:3). When no more change was observed (after ~16 h) a part of the solvent was removed by rv, the remaining one diluted with AcOEt, washed twice with 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with AcOEt, the comb. org. layers dried over $MgSO_4$, filtered and dried in rv and hv. 1.01 g of the crude product were obtained, adsorbed at SiO_2 and

purified over SiO_2 (H:AcOEt 1:1 to 2:3 and 1:2). 0.52 g (0.45 mmol, 74%) Boc-[TSer(OBn)]$_4$-OMe (**95**) were isolated.

R_f(H/AcOEt 1:3): 0.55; R_f(H/AcOEt 1:2): 0.35
IR (KBr): 3926*w*; 3760*m*; 3361*s*; 3236*s*; 3121*w*; 3031*w*; 2921*w*; 2863*w*; 2029*m*; 1775*m*; 1714*s*; 1635*s*; 1615*s*; 1572*m*; 1534*s*; 1490*s*; 1454*m*; 1391*w*; 1365*m*; 1245*s*; 1215*s*; 1167*s*; 1100*s*; 1028*s*; 914*w*; 845*w*; 814*w*; 781*w*; 753*m*; 698*m*; 642*s*; 508*m*.



95

^{1}H-NMR (300 MHz, DMSO): Mixture of diastereoisomers: 8.97 – 8.90 (*m*, 3 H, Amide-NH); 8.48; 8.47; 8.35; 8.35; 8.33 (5 *s*, 4 H, Thiazole-CH); 7.35 – 7.22 (*m*, 20 H, arom. CH); 5.70 – 5.60 (*m*, 4 H, NCH); 5.15 (br. *d*, ^3J = 4.5, 1 H, Carbamat-NH); 4.58 – 4.54 (*m*, 8 H, 4 OCH_2Ph); 4.16 – 3.93 (*m*, 7 H, 4 CH_2); 3.86 – 3.80 (*m*, 1 H, CH_2); 3.84 (*s*, 3 H, OCH_3); 1.42 (*s*, 9 H, $OC(CH_3)_3$).

^{13}C-NMR (100 MHz, DMSO): 171.14; 171.10; 170.92 (3 *s*, 4 C, 4 Thiazole NCS); 161.19; 160.63; 160.60; 160.49 (4 *s*, 4 C, COOMe, 3 Amide-CO); 155.47 (*s*, 1 C, Carbamate-CO); 148.64; 145.49 (2 *s*, 4 C, Thiazole-C); 138.04; 137.91 (2 *s*, 4 C, arom. C); 129.51; 129.16; 128.29; 128.23; 127.57; 127.55; 127.52; 125.45; 125.31 (9 *d*, 24 C, 20 arom. CH, 4 Thiazole-CH); 78.87 (*s*, 1 C, $OCMe_3$); 72.23; 72.17; 71.14; 70.56; 70.41 (5 *t*, 8 C, 4 CH_2OCH_2); 53.08 (*d*, 1 C, NCH); 52.03 (*q*, 1 C, OCH_3); 51.42; 51.38; 51.20 (3 *d*, 3 C, 3 NCH); 28.18 (*q*, 3 C, $OC(CH_3)_3$).

MS (ESI, MeOH + NaI): 1195.6 (100, $[M + Na]^+$).

8.2.4 Macrolactamization to **60**

8.2.4.1 Synthesis of **100**

0.17 g of **95** (0.15 mmol) were dissolved in 6 ml MeOH at rt. 18 mg of LiOH (0.77 mmol, 5 equiv.) were dissolved in 2 ml H_2O and added to the solution of **95**. A part of the starting material precipitated again. The reaction was monitored on TLC (H:AcOEt 1:3) and in HPLC (reversed phase). After completion a part of the solvent was removed by rv. The remaining solution was acidified with 1 M $NaHSO_4$, whereas a colourless precipitate forms which is twice extracted with AcOEt. The org. layers were washed with 0.1 M citric acid in brine, combined, dried over $MgSO_4$, filtered and dried in rv and hv. The so isolated **100** was directly submitted to deprotection on the *N*-terminus.

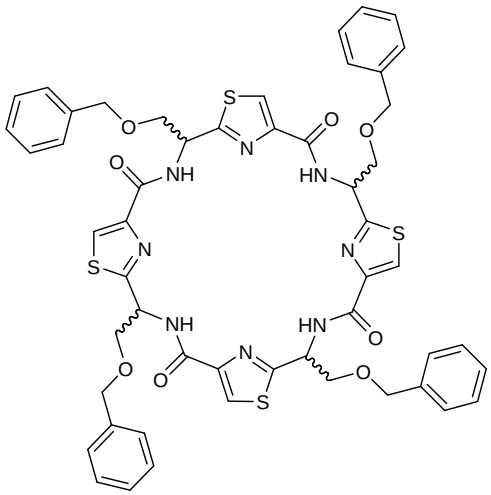
8.2.4.2 Synthesis of **101**

0.15 mmol of **100** were dissolved in 6 ml CH_2Cl_2 under N_2 at rt and 6 ml TFA were added. Reaction was monitored in HPLC (reversed phase). The solvent was removed under reduced pressure freezing it out in a cooling trap and the remaining oil was dried in hv. It was then dissolved in MeOH and stirred at rt under N_2 for 16 h over Amberlyst-A26 in it's Cl^--form, then filtered, dried in rv and hv. 0.16 g **101** were isolated and directly used for macrolactamisation.

8.2.4.2 Synthesis of **60**

0.28 mmol of **101** were dissolved in 175 ml DMF (dry, solvent system, concentration of 1.5 mM), 300 µl of NMM (2.70 mmol, 10 equiv.) were added, the pH tested to be basic and 714 mg of PyBOP (1.37 mmol, 5 equiv.) were added. The solution was stirred at rt under N_2. The evolution of product **60** was monitored on TLC (H:AcOEt 1:3) and HPLC (reversed phase). When no more change was observed a part of the solvent was removed by rv, the remaining oil diluted with AcOEt, washed twice with 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with AcOEt, the comb. org. layers dried over $MgSO_4$, filtered and dried in rv and hv. The crude product was adsorbed at SiO_2 and chromatographically purified on SiO_2 (H:AcOEt 1:1 to 1:2 and 1:2). 154 mg (148 µmol, 54%) 3,11,19,27-tetra[(benzyloxy)mehtyl]-1,9,17,25-tetroxo-2,10,18,26-tetraza[3.$_4$](2,4)1,3-thiazolophan (**60**) could be isolated.

Melting temperature: solidified foam, 124.3 – 126.9°C
R_f(H/AcOEt 1:5): 0.4
IR (KBr): 3934*w*; 3892*w*; 3876*w*; 3859*w*; 3844*w*; 3826*w*; 3808*w*; 3761*w*; 3717*m*; 3695*m*; 3681*m*; 3655*m*; 3413*s*; 3236*s*; 3031*w*; 2921*w*; 2860*w*; 2030*m*; 1640*s*; 1615*s*; 1538*s*; 1493*s*; 1453*m*; 1363*m*; 1247*s*; 1155*s*; 1106*s*; 1028*m*; 913*w*; 847*m*; 750*m*; 698*m*; 627*s*; 491*m*.



60

^{1}H-NMR (300 MHz, DMSO + 1 Tr MeOD): Mixture of diastereoisomers: 8.40; 8.38; 8.36; 8.35; 8.34; 8.29; 8.26 (7 *s*, 4 H, Thiazole-CH); 7.30 – 7.14 (*m*, 20 H, arom. CH); 5.71 – 5.53 (*m*, 4 H, NCH); 4.57 – 4.45 (*m*, 8 H, 4 OCH_2Ph); 4.06 – 3.82 (*m*, 8 H, 4 CH_2).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 168.60; 168.10; 168.05; 167.91; 167.89; 167.80; 167.65 (7 *s*, 4 C, 4 Thiazole-NCS); 160.06; 160.02; 159.91; 159.86; 159.72; 159.67 (6 *s*, 4 C, 4 Amide-CO); 148.35; 148.30; 148.08; 148.05; 147.89; 147.65; 147.42 (7 *s*, 4 C, 4 Thiazole-C); 138.00; 137.89; 137.80; 137.72; 137.66; 137.63; 137.58 (7 *s*, 4 C, 4 arom. C); 128.24; 128.20; 128.14; 128.04; 127.67; 127.59; 127.56; 127.49; 127.44; 127.35; 127.30; 127.26; 126.65; 126.52; 125.80; 125.50; 125.02 (17 *d*, 24 C, 20 arom. CH, 4 Thiazole-CH); 72.33; 72.25; 72.14; 72.04; 71.98 (2 *t*, 4 C, 4 OCH_2Ph); 70.78; 70.70; 70.54; 70.05; 69.72; 69.55 (6 *t*, 4 C, 4 CH_2); 51.23; 50.79; 50.43; 49.92; 49.41; 49.27; 48.51 (7 *d*, 4 C, 4 NCH).

MS (ESI, MeOH + NaI): 1213.1 (5); 1063.4 (100, [*M* + Na]$^+$).

8.2.5 Investigations towards the synthesis of thiazole units

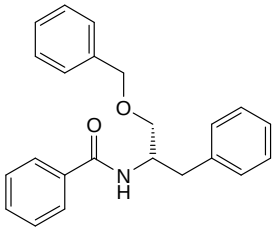
8.2.5.1 Reactions using model compounds

8.2.5.1.1 Reactions using **63**

a. Benzylation of **63** to synthesize **102**

0.50 g of **63** (1.97 mmol) were suspended in 20 ml dry DME (solvent system) and 178 mg of NaH (55-65% on parafin, ~4.5 mmol, 2.2 equiv.) were added under N_2. 250 µl of benzylchloride (2.17 mmol, 1 equiv.) were added. After four hours, the suspension was heated up to 100°C. Completion of the reaction could be monitored on TLC (H:AcOEt 1:1). The solution was then diluted with TBME, washed once with 1 M $NaHSO_4$, once with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME, the comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv and v. The product was filtered over SiO_2, first washing out the parafin with H:AcOEt 4:1, then product **102** with H:AcOEt 1:1. The product was recrystallized from hot H:AcOEt 3:1. 252 mg (730 µmol, 37%) of the pure colourless crystalline *N*-(1-benzyloxy-3-phenylpropan-2-yl)benzamide (**102**) were isolated.

R_f(H/AcOEt 5:1): 0.10



102

^{1}H-NMR (300 MHz, $CDCl_3$): 7.71 – 7.69; 7.51 – 7.16 (2 *m*, 15 arom. CH); 6.49 (br. *d*, ^3J = 8.2, 1 H, NH); 4.52 (*d*, ^3J = 2.3, 2 H, OCH_2Ph); 4.51 – 4.45 (*m*, 1 H, NCH); 3.5 (*d*, ^3J = 3.6, 2 H, OCHC); 3.05 (*dd*, ^2J = 13.4, ^3J = 6.1, 1 H, CCH_2Ph); 2.97 (*dd*, ^2J = 13.4, ^3J = 8.5, 1 H, CCH_2Ph).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 167.00 (*s*, 1 C, CONH); 138.16; 138.13; 134.87 (3 *s*, 3 C, arom. C); 131.55; 129.61; 128.66; 128.63; 128.05; 128.03; 127.06; 126.64 (8 *d*, 15 C, arom. CH); 73.48 (*t*, 1 C, OCH_2Ph); 69.79 (*t*, 1 C, OCH_2C); 50.91 (*d*, 1 C, NCH); 37.67 (*t*, CCH_2Ph).

MS (ESI, MeOH / CH_2Cl_2 3:1 + NaI): 368.2 (100, $[M + Na]^+$).

b. Debenzylation of **102** to synthesize **63**

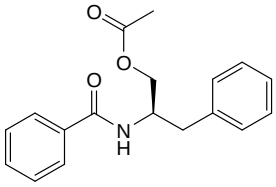
20 mg of **102** (58 µmol) were dissolved in 2 ml CH_2Cl_2 (*puriss.*), 47 ml of anisole (430 µmol, 7.5 equiv.) and 47 µg of $AlCl_3$ (anhydrous, 50 µmol, 6 equiv.) were added. The mixture was stirred overnight under N_2 at rt. The conversion was monitored on TLC (H:AcOEt 1:1). The solution was diluted with AcOEt, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv and hv. 9.0 mg **63** (35 mmol, 60%) were isolated.

c. Synthesis of **148** by acetylation

255 mg of **63** (1.00 mmol) were dissolved in 2.5 ml pyridine and cooled down to 0°C. 0.24 ml of Ac_2O (2.5 mmol, 2.5 equiv.) were added and the mixture was stirred under N_2 for 5 h, slowly warming up to rt. The reaction was monitored on TLC (H:AcOEt 1:1). The rest of Ac_2O was quenched with MeOH and 1 M aq. HCl. A colourless precipitate formed, which was extracted with TBME. The org. layers were washed three times with 1 M HCl, once with H_2O and once with brine. The comb. org.

layers were dried over $MgSO_4$, filtered and dried in rv. 284 mg (955 μmol, 96%) of the pure *N*-(1-acetoxy-3-phenylpropan-2-yl)benzamide (**148**) were isolated as a colourless solid.

R_f(H/AcOEt 1:1): 0.7



148

^{1}H-NMR (300 MHz, $CDCl_3$): 7.73 – 7.21 (*m*, 10 H, arom. CH); 6.38 (br. *d*, ^3J = 7.9, 1 H, NH); 4.64 – 4.60 (*m*, 1 H, NCH); 4.34 (*dd*, ^2J = 11.5, ^3J = 5.9, 1 H, OCH_2); 4.14 (*dd*, ^2J = 11.5, ^3J = 4.2, 1 H, OCH_2); 3.06 (*dd*, ^2J = 13.7, ^3J = 5.9, 1 H, CH_2Ph); 2.90 (*dd*, ^2J = 13.7, ^3J = 7.9, 1 H, CH_2Ph); 2.09 (*s*, 3 H, $(CO)CH_3$).

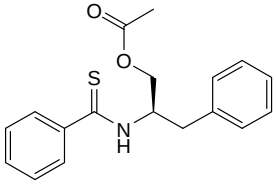
^{13}C-NMR (75.5 MHz, $CDCl_3$): 171.31 (*s*, 1 C, COO); 166.93 (*s*, 1 C, CONH); 136.85; 134.26 (2 *s*, 2 C, arom. C); 131.46; 129.20; 128.59; 128.51; 126.75 (5 *d*, 10 C, arom. CH); 64.74 (*t*, 1 C, OCH_2); 50.24 (*d*, 1 C, NCH); 37.42 (*t*, 1 C, CH_2Ph); 20.74 (*q*, 1 C, CH_3).

MS ($CHCl_3$ / MeOH 4:6 + NaI): 320.1 (100, $[M + Na]^+$); 260.0 (3, $[M – AcOH + Na]^+$); 238.0 (3, $[M – AcOH + H]^+$).

d. Synthesis of **149** by thionation

151 mg of **148** (508 μmol) were dissolved in 10 ml dry toluene (*puriss. p.a.*) in a dry flask. 123 mg of Lawesson reagent (304 mmol, 0.6 equiv.) were added and the mixture was heated to reflux for 150 min. The progress of the reaction was monitored on TLC (H:AcOEt 5:1). Toluene was then removed by rv and the remaining oily material chromatographically purified on SiO_2 (H:AcOEt 8:1 to 5:1). 149 mg of the 3-phenyl-2-(thiobenzoylamino)prop-1-yl acetate (**149**) (475 μmol, 94%) were isolated.

R_f(H/AcOEt 5:1): 0.45



149

^{1}H-NMR (300 MHz, $CDCl_3$): 7.97 (br. *d*, ^3J = 6.4, 1 H, NH); 7.73 – 7.24 (*m*, 10 H, arom. CH); 5.20 – 5.15 (*m*, 1 H, NCH); 4.33 (*dd*, ^2J = 11.9, ^3J = 5.4, 1 H, OCH_2); 4.26 (*dd*, ^2J = 11.9, ^3J = 3.7, 1 H, OCH_2); 3.28 (*dd*, ^2J = 13.6, ^3J = 5.2, 1 H, CH_2Ph); 2.94 (*dd*, ^2J = 13.6, ^3J = 8.8, 1 H, CH_2Ph); 2.12 (*s*, 3 H, $(CO)CH_3$).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 198.87 (*s*, 1 C, CSNH); 171.56 (*s*, 1 C, COO); 141.59; 136.48 (2 *s*, 2 C, arom. C); 131.07; 129.17; 128.68; 128.44; 126.93; 126.56 (6 *d*, 10 C, arom. CH); 63.97 (*t*, 1 C, OCH_2); 56.40 (*d*, 1 C, NCH); 35.66 (*t*, 1 C, CH_2Ph); 20.75 (*q*, 1 C, CH_3).

MS (ESI, MeOH): 336.2 (5, $[M + Na]^+$); 314.2 (13, $[M + H]^+$); 272.1 (5); 254.1 (100, $[M – CH_3CO_2H + H]^+$).

e. Oxazolin opening using H_2S to synthesize β-hydroxythioamide **110**

1.4 ml of Et_3N (10 mmol, 10 equiv.), 1.20 g of $Na_2S.9H_2O$ (5.00 mmol, 5 equiv.) and 0.6 ml AcOH (10 mmol, 10 equiv.) were added to 5 ml cold MeOH under N_2 flow. 225 mg of **72** (948 μmol) were dissolved in another 5 ml MeOH and dropped into the solution of H_2S. The mixture turned slightly yellow and was slowly let warm up to rt while stirring. The reaction was monitored on TLC (H:AcOEt 5:1). After 24 h no change was observed and 5 ml of Et_3N were added. 4 d later the solution was diluted

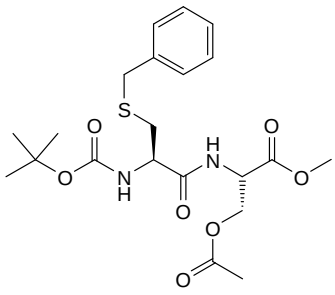
with TBME, washed three times with sat. $NaHCO_3$, once with 0.1 M citric acid, once with H_2O and once with brine. All aq. layers were back extracted with TBME, the comb. org. layers dried over $MgSO_4$, filtered and solvent removed by rv. 235 mg of the crude mixture were isolated and chromatographically purified on SiO_2. 59% of the starting material **72** and 92 mg of pure **110** (0.34 mmol, 36%), identified by ^{13}C-NMR with the characteristic thioamide-C at 198.92 ppm, were isolated.[5]

8.2.5.1.2 Reactions using **106**

a. Synthesis of **107** by acetylation

1.00 g of **106** (2.43 mmol) were dissolved in 10 ml pyridine (*puriss. p.a.*) under N_2. The solution was cooled down to 0°C and 570 µl of Ac_2O (6.03 mmol, 2.5 equiv.) were added. The solution was stirred overnight, slowly warming up to rt. TLC (H:AcOEt 1:1) showed quantitative conversion. The solution was diluted with 100 ml 1 M HCl, a colourless solid precipitated which was extracted with TBME. The org. layer was washed again twice with 1 M HCl, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME. Comb. org. layers were dried over $MgSO_4$, filtered and dried in rv. 984 mg crude product were recrystallized from EtOH. 715 mg (1.57 mmol, 65%) pure Boc-Cys(SBn)-Ser(OAc)-OMe (**107**) were isolated as a colourless solid.

R_f(H/AcOEt 1:2): 0.20


107

^{1}H-NMR (300 MHz, $CDCl_3$): 7.36 – 7.24 (*m*, 5 H, arom. CH); 7.11 (br. *d*, ^3J = 7.6, 1 H, Amide-NH); 5.29 (br. *s*, 1 H, Carbamat-NH); 4.80 (*dt*, ^3J = 8.1, ^3J = 3.8, 1 H, *N*-terminal NCH); 4.45 (*dd*, ^2J = 11.4, ^3J = 4.1, 1 H, OCH_2); 4.36 (*dd*, ^2J = 11.4, ^3J = 3.3, 1 H, OCH_2); 4.28 (br. *d*, ^3J = 6.1, 1 H, *C*-terminal NCH); 3.77 (*s*, 5 H, OCH_3 & SCH_2Ph); 2.88 (*dd*, ^2J = 14.0, ^3J = 5.9, 1 H, SCH_2); 2.77 (*dd*, ^2J = 14.0, ^3J = 6.5, 1 H, SCH_2); 2.30 (*s*, 3 H, $(CO)CH_3$); 1.46 (*s*, 9 H, $OC(CH_3)_3$).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 170.57; 170.35; 169.30 (3 *s*, 3 C, 2 COO, 1 CONH); 155.16 (*s*, 1 C, Carbamat OCONH); 137.69 (*s*, 1 C, arom. C); 128.90; 128.50 (2 *d*, 4 C, arom. *o*-, *m*-CH); 127.15 (*d*, 1 C, arom. *p*-CH); 80.37 (*s*, 1 C, $OCMe_3$); 63.43 (*t*, 1 C, OCH_2); 53.66; 52.74 (2 *d*, 2 C, NCH); 51.80 (*q*, 1 C, OCH_3); 36.41; 33.52 (2 *t*, 2 C, CH_2SCH_2); 28.16 (*q*, 3 C, $OC(CH_3)_3$); 20.50 (*q*, 1 C, $(CO)CH_3$).

MS (ESI, CH_2Cl_2:MeOH 1:3 + NaI): 931.5 (10, $[2M + Na]^+$); 709.4 (6); 493.3 (17, $[M + K]^+$); 477.3 (100, $[M + Na]^+$); 382.2 (10, $[M – OCMe_3]^+$); 355.2 (33, $[M – CO_2CMe_3 + 2 H]^+$); 338.2 (10).

b. Synthesis of **108** by thionation

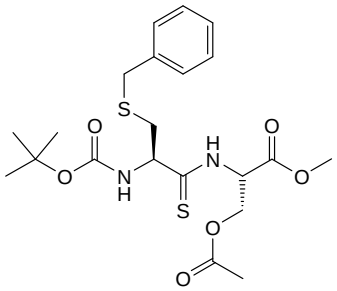
454 mg of **107** (1.00 mmol) were dissolved in 5 ml boiling toluene and 201 mg of Lawesson reagent (0.50 mmol, 0.5 equiv.) were added and the solution was refluxed for 2 h. The progress of the reaction was monitored on TLC (H:AcOEt 3:1), two products were formed. Toluene was partially removed, the remaining oil diluted with AcOEt, washed once with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, comb. org. layers dried over

[5] See more efficient procedure in section 8.2.5.1.3 a.

$MgSO_4$, filtered and dried in rv. The 642 mg of the crude product were adsorbed at SiO_2 and chromatographically purified on SiO_2 (H:AcOEt 5:1 to 2:1). 9 mg of the pure thioamide **108a** (0.02 mmol, 2%), 362 mg of the mixture and 186 mg of thiazoline **108b** (395 µmol, 40%) were isolated.

108a: R_f(H/AcOEt 3:1): 0.7; R_f(H/AcOEt 4:1): 0.45
108b: R_f(H/AcOEt 3:1): 0.5; R_f(H/AcOEt 4:1): 0.25

108a: *^{1}H-NMR (300 MHz, $CDCl_3$)*: 8.70 (br. *d*, ^3J = 6.8, 1 H, Thioamide-NH); 7.36 – 7.25 (*m*, 5 H, arom. CH); 5.37 (br. *s*, 1 H, Carbamat-NH); 5.34 (*dt*, ^3J = 7.2, ^3J = 3.5, 1 H, *N*-terminal NCH); 4.59 (*dd*, ^2J = 11.7, ^3J = 3.3, 1 H, OCH_2); 4.52 (*dd*, ^2J = 11.7, ^3J = 3.5, 1 H, OCH_2); 4.51 (*dd*, ^3J = 13.3, ^3J = 6.5, 1 H, *C*-terminal NCH); 3.80 (*s*, 3 H, OCH_3); 3.76 (*s*, 2 H, SCH_2Ph); 2.94 (*d*, ^2J = 6.2, 2 H, SCH_2); 2.03 (*s*, 3 H, $(CO)CH_3$); 1.46 (*s*, 9 H, $OC(CH_3)_3$).



108a

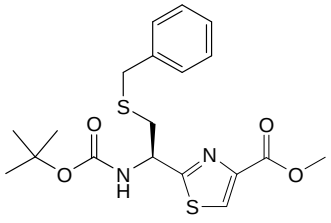
MS (ESI, CH_2Cl_2:MeOH 1:3 + NaI): 509.2 (29, $[M_a + K]^+$); 493.2 (100, $[M_a + Na]^+$).

c. Synthesis of thiazole **109**

167 mg of **108b**[6] (407 µmol) were dissolved in 3 ml CH_2Cl_2 under N_2 at rt. 123 µl of DBU (814 µmol, 2 equiv.) and 42 µl of $BrCCl_3$ (427 µmol, 1.05 equiv.) were added. The solution was stirred for 1 h and turned dark. The completion of the reaction was monitored on TLC (H:AcOEt 4:1). The solution was diluted with TBME, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME, the comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. 137 mg of the crude product were chromatographically purified on SiO_2 (H:AcOEt 4:1, 3:1, 2:1). 112 mg pure methyl 2-{2*S*-2-(benzylsulphanyl)-1-[(*tert*-butoxycarbonyl)amino]ethyl}-thiazole-4-carboxylate (L-Boc-TCys(SBn)-Ome, **109**) (274 µmol, 69%) were isolated as a slightly yellow oil.

R_f(H/AcOEt 4:1): 0.35

^{1}H-NMR (300 MHz, $CDCl_3$): 8.12 (*s*, 1 H, Thioazole-CH); 7.33 – 7.23 (*m*, 5 H, arom. CH); 5.53 (br. *s*, 1 H, Carbamat-NH); 5.20 (br *d*, ^3J = 6.8, 1 H, NCH); 3.94 (*s*, 3 H, OCH_3); 3.59 (*s*, 2 H, SCH_2Ph); 3.14 (*dd*, ^2J = 13.9, ^3J = 5.9, 1 H, SCH_2); 3.02 (*dd*, ^2J = 13.9, ^3J = 6.0, 1 H, SCH_2); 1.45 (*s*, 9 H, $OC(CH_3)_3$).



109

MS (ESI, CH_2Cl_2:MeOH 1:3 + NaI): 447.1 (4, $[M + K]^+$); 431.1 (100, $[M + Na]^+$).

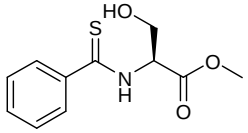
8.2.5.1.3 Reactions using **64**

a. Oxazolin opening using H_2S to synthesize β-hydroxythioamide **111**

50 mg of **75** (244 µmol) were dissolved in 2 ml MeOH and 2 ml of Et_3N. During 20 min H_2S was bubbled through the solution at rt[210, 211]. Gas flow was then stopped and the solution was stirred at rt for 2 h. The progress of the reaction was monitored

[6] It is also possible to use a mixture of **108a** and **108b**, the conversion to **109** is performing equally good.

on TLC (H:AcOEt 2:1). Solution was diluted with AcOEt, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. Aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. 66 mg of the crude methyl 2-(thiobenzoylamino)-3-hydroxypropanoate (**111**) was obtained and directly converted into **113** usind DAST cyclisation and DBU, $BrCCl_3$ oxidation.



111

R_f(H/AcOEt 3:1): 0.45

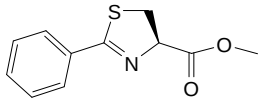
b. Synthesis of thiazoline **112** using **111**

66 mg of crude material **111** (containing max. 244 μmol) were dissolved in 4 ml CH_2Cl_2 (*puriss*. *p.a.*) and cooled down to -10℃ under N_2. 38 μl of DAST (0.29 mmol, 1.2 equiv.) were added. Reaction was monitored on TLC (H:AcOEt 3:1). After two hours another 9 μl of DAST (0.07 mmol, 0.3 equiv.) were added. 20 min later no more **111** was detected. The solution was diluted with CH_2Cl_2, washed with sat. $NaHCO_3$ and brine, aq. layers were back extracted, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. 57 mg of the crude methyl (4*S*)-2-phenyl-1,3-thiazoline-4-carboxylate (**112**) could be isolated and directly used for oxidation.

c. Synthesis of thiazoline **112** using **64**

41 mg of **64** (0.18 mmol) were dissolved in 2.5 ml dry pyridine and 82 mg of P_2S_5 (0.37 mmol, 2 equiv.) were added[212]. The mixture was heated to reflux (oil bath 150℃). According to TLC (H:AcOEt 1:1) conversion was complete after 2 h. Solvent was removed under reduced pressure freezing it out in a cooling trap. The remaining oil was chromatographically purified on SiO_2 (H:AcOEt 2:1) and 28 mg of the pure methyl (4*S*)-2-phenyl-1,3-thiazoline-4-carboxylate (**112**) (0.13 mmol, 69%) were isolated as a yellow oil.

R_f(H/AcOEt 3:1): 0.55



112

^{1}H-NMR (300 MHz, $CDCl_3$): 7.88 – 7.85 (*m*, 2 H, 2 arom. CH *meta*); 7.50 – 7.37 (*m*, 3 H, 3 arom. CH *ortho*, *para*); 5.29 (*t*, ^3J = 9.1, 1 H, N-CH); 3.83 (*s*, 3 H, OCH_3); 3.69 (*dd*, ^2J = 26.1, ^3J = 11.2, 1 H, SCH_2); 3.66 (*dd*, ^2J = 26.6, ^3J = 11.2, 1 H, SCH_2).

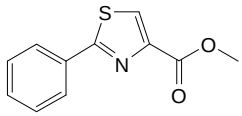
^{13}C-NMR (75.5 MHz, $CDCl_3$): 171.53; 171.18 (2 *s*, COOMe & CNS); 132.87 (*s*, 1 arom. C); 131.88 (*d*, 2 arom. CH(*meta*)); 128.82; 128.69 (2 *d*, 3 arom. CH(*ortho*, *para*)); 78.69 (*d*, 1 C, N-CH); 52.97 (*q*, 1 C, O-CH_3); 35.57 (*t*, 1 C, S-CH_2).

MS (MeOH + NaI): 243.9 (100, $[M + Na]^+$); 222.0 (5, $[M + H]^+$).

d. Synthesis of thiazole **113** by oxidation

53 mg of **112** (0.25 mmol) were dissolved in 4 ml CH_2Cl_2 (*puriss. p.a.*) and cooled in an ice bath to 0℃. 74 μl of DBU (0.49 mmol, 2 equiv.) and 29 μl of $BrCCl_3$ (0.29 mmol, 1.2 equiv.) were added. Solution was let warm up to rt while stirring under N_2. After 2 h TLC (H:AcOEt 3:1) showed complete conversion. The solution was diluted with CH_2Cl_2, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. Aq. layers were back extracted with CH_2Cl_2, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. The crude **113** was chromatographically purified on SiO_2 (H:AcOEt 5:1) and 37 mg of the pure methyl (4*S*)-2-phenyl-1,3-thiazole-4-carboxylate (**113**) (0.17 mmol, 69%) were isolated as a slightly yellow solid.

R_f(H/AcOEt 3:1): 0.55



113

^{1}H-NMR (300 MHz, $CDCl_3$): 8.18 (*s*, 1 H, Thiazole-CH); 8.03 – 7.99 (*m*, 2 H, 2 arom. *m*-CH); 7.50 – 7.37 (*m*, 3 H, 3 arom. *o*-/*p*-CH); 3.99 (*s*, 3 H, OCH_3).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 169.03 (*s*, 1 C, COO); 161.95 (*s*, 1 C, Thiazole-C^2); 147.68 (*s*, 1 C, Thiazole-C^4); 132.72 (*s*, 1 C, arom. C); 130.76; 129.00; 127.36; 126.98 (4 *d*, 6 C, 5 arom. CH, Thiazole-C^5H); 52.52 (*q*, 1 C, OCH_3).

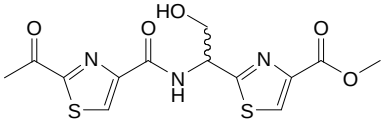
MS (MeOH + NaI): 241.9 (100, $[M + Na]^+$).

8.2.5.1.4 Reactions using **99**

a. Debenzylation to give β-hydroxyamide **104**

117 mg of **99** (263 µmol) were dissolved in 10 ml CH_2Cl_2 (*puriss. p.a.* o.m.s.) and cooled down to 0℃. 0.5 ml of Tf_2O (3 mmol, 11 equiv.) and 376 µl of $BF_3.Et_2O$ (2.63 mmol, 10 equiv.) were added and the solution was slowly let warm up to rt while stirring under N_2 overnight. Reaction was monitored on TLC (H:AcOEt 1:3). The suspension was diluted with AcOEt, washed once with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. The crude material was adsorbed at SiO_2 and chromatographically purified on SiO_2. 40 mg of the starting material **99** (90 µmol, 34%) and 56 mg of methyl 2-{1-[(2-acetyl-1,3-thiazole-4-yl)carbonylamino]-2-hydroxyethyl}-1,3-thiazole-4-carboxylate (**104**) (0.16 mmol, 60%) were isolated.

R_f(H/AcOEt 1:3): 0.2



104

^{1}H-NMR (300 MHz, $CDCl_3$): 8.46 (*s*, 1 H, Thiazole-CH); 8.36 (br. *d*, ^3J = 8.2, 1 H, NH); 8.16 (*s*, 1 H, Thiazole-CH); 5.58 (*dt*, ^3J = 8.2, ^3J = 4.0, 1 H, NCH); 4.47 (*dd*, ^2J = 11.4, ^3J = 3.8, 1 H, OCH_2); 4.13 (*dd*, ^2J = 11.9, ^3J = 4.2, 1 H, OCH_2); 3.94 (*s*, 3 H, OCH_3); 2.74 (*s*, 3 H, $(CO)CH_3$).

MS (ESI, MeOH): 378.1 (100, $[M + Na]^+$).

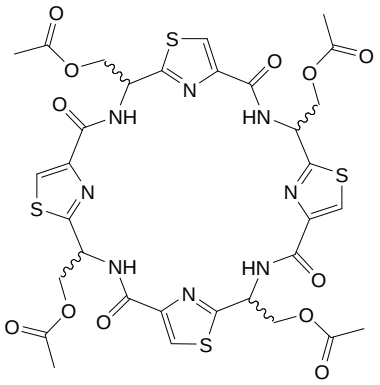
8.2.5.2 Reactions on the macrocycle

8.2.5.2.1 Debenzylation to give tetraacetoxy macrocycle **105**

397 mg of **60** (381 µmol) were dissolved in a mixture of 40 ml CH_2Cl_2 (*puriss. p.a.*) and 40 ml of Ac_2O. 2.4 ml of $BF_3.Et_2O$ (~7 M, 17 mmol, 4 x 10 equiv.) were added and the solution was stirred under N_2 at rt overnight. The progress of the reaction was monitored on TLC ($CHCl_3$:MeOH 12:1). The solvent was then removed in rv, the remaining crude material adsorbed at SiO_2 and chromatographically purified on SiO_2 using CH_2Cl_2:MeOH 40:1 as solvent. 233 mg 3,11,19,27-tetra[(acetoxy)methyl]-1,9,17,25-tetroxo-2,10,18,26-tetraza[3.4](2,4)1,3-thiazolophan (**105**) (274 mmol, 72%) were isolated.

Melting temperature: 261.3 – 269.3℃ (amorphous solid)

R_f(H/AcOEt 1:5): 0.25
IR (KBr): 3928*w*; 3860*w*; 3375*s*; 3236*s*; 2927*w*; 2854*w*; 2030*m*; 1744*m*; 1638*s*; 1616*s*; 1543*m*; 1492*m*; 1365*w*; 1224*m*; 1157*m*; 1050*m*; 806*w*; 767*w*; 631*s*; 500*m*.



105

^{1}H-NMR (300 MHz, DMSO): mixture of diastereoisomers: 8.93 – 8.67 (*m*, 4 H, NH); 8.45; 8.43; 8.42; 8.40; 8.38; 8.33 (6 *s*, 4 H, Thiazole-CH); 5.75 – 5.61 (*m*, 4 H, NCH); 4.87 – 4.41 (*m*, 8 H, CH_2); 2.01; 2.00; 1.99; 1.95; 1.95; 1.92; 1.88 (7 *s*, 12 H, (CO)CH_3).

^{13}C-NMR (100 MHz, DMSO): mixture of diastereoisomers: 170.26; 170.23; 170.13; 170.16 (4 *s*, 4 C, COO); 167.03; 167.00; 166.81; 166.79; 166.78 (5 *s*, 4 C, CONH); 160.23; 160.12; 159.93; 159.79 (4 *s*, 4 C, Thiazole-C^2); 148.44; 148.18; 147.97; 147.92; 147.69 (5 *s*, 4 C, Thiazole-C^4); 126.91; 126.88; 126.46; 126.25; 125.86 (5 *d*, 4 C, Thiazole-C^5H); 64.44; 64.27; 64.11; 63.76; 63.42 (5 *t*, 4 C, CH_2); 50.37; 49.39; 49.33; 49.07; 48.38 (5 *d*, 4 C, NCH); 20.57 (*q*, 4 C, CH_3).

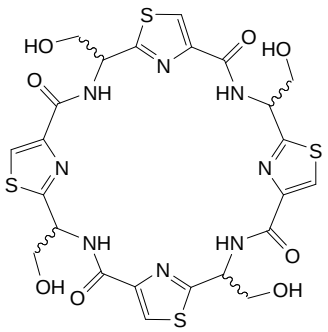
MS (ESI, MeOH:$CHCl_3$ 1:1 + NaI): 919.3 (6, [*M* – $COCH_3$ + C_7H_7 + Na]$^+$); 871.3 (100, [*M* + Na]$^+$); 685.5 (13, [*M* – CH_3CO_2H – 3 CH_3CO + 3 H + Na]$^+$); 663.5 (5, [*M* – CH_3CO_2H – 3 CH_3CO + 4 H]$^+$); 413.4 (8); 304.3 (15).

8.2.5.2.2 Hydrolysis of **105** to tetraalcohol **59**

200 mg of **105** (236 μmol) were suspended in 20 ml MeCN, 20 ml MeOH and 5 ml H_2O and heated up to 45℃. 52 mg of LiOH (2.2 mmol, 1 0 equiv.) were dissolved in 15 ml H_2O. This solution was added over 5 min to the hot suspension of **105**. The suspension was further stirred for 3 min, during which time all material dissolved. Then 2.2 ml 1 M HCl were added and the pH was adjusted to about 7 by adding some more drops of 1 M HCl. The solvent was removed in rv, the remaining material adsorbed at SiO_2 and the product chromatographically purified on SiO_2 ($CHCl_3$: MeOH 10:1 to 5:1). 143 mg of the colourless solid **59** (210 μmol, 89%) were isolated.

8.2.5.2.3 Debenzylation to tetraalcohol **59**

504 mg of **60** (484 μmol) were dissolved in 50 ml $ClCH_2CH_2Cl$. 1.7 ml of Tf_2O (19 mmol, 4 x 10 equiv.) and 2.2 ml $BF_3.Et_2O$ (7 M, 15 mmol, 4 x 8 equiv.) were added. The reaction was monitored on TLC ($CHCl_3$:MeOH 5:1) and stopped after 4 h by cooling down to 0℃ in an ice bath, adding little ice water, diluting after 5 min with MeOH to get one layer and adjusting to neutral pH with sat. Na_2CO_3. The solvent was then removed in rv, the remaining solid suspended in a 1:1 mixture of $CHCl_3$ and MeOH, filtered and adsorbed to SiO_2. After column chromatography (H:AcOEt:MeOH 1:5:1) 152 mg 3,11,19,27-tetra(hydroxymehtyl)-1,9,17,25-tetroxo-2,10,18,26-tetraza[3.4] (2,4)1,3-thiazolophan (**59**) (223 mmol, 46%) were isolated.



59

R_f($CHCl_3$/MeOH 5:1): 0.23

^{1}H-NMR (300 MHz, DMSO): mixture of diastereoisomers: 8.73 – 8.40 (*m*, 4 H, NH); 8.34; 8.33; 8.32; 8.31; 8.30; 8.29; 8.26 (7 *s*, 4 H, thiazole-CH); 5.48 – 5.29 (*m*, 4 H, NCH); 4.11 – 3.72 (*m*, 8 H, CH_2).

MS (ESI, MeOH + NaI): 703.2 (100, $[M + Na]^+$).

8.2.5.2.4 Optimized procedure for thionation of **105**

8 mg of **105** (9 µmol) were suspended in 2 ml dry DME (solv. system). A spatula of LiOTf and one of Lawesson reagent were added and the suspension was stirred under reflux for 7 h. It was then diluted with AcOEt, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. An ESI MS of the crude material revealed some of the thioamide product.

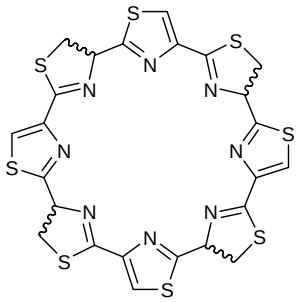
MS (ESI, $CHCl_3$/MeOH 3:1 + NaI): 933.2 (10, $[M + Na]^+$); 871.3 (100, $[M_{\mathbf{105}} + Na]^+$).

8.2.5.2.5 Optimized procedure for the formation of **115** from **59**

50 mg of **59** (73 µmol) were dissolved in 6 ml dry pyridine and 135 mg of P_2S_5 (607 µmol, 4 x 2 equiv.) were added. The suspension, which solubilized at higher temperatures, was heated to 150°C under N_2 and stirred at this temperature for 5 h. The pyridine was removed then under reduced pressure freezing it out in a cooling trap, the remaining material was suspended using sonication in a mixture of AcOEt, $CHCl_3$ and MeOH and filtered. The solvent was removed in rv and the residue again dissolved in $CHCl_3$ and MeOH, adsorbed at SiO_2 and chromatographically purified on SiO_2 (CH_2Cl_2:MeOH 25:2). 10 mg of fractions containing to a big extent the desired [0.$_4$]{(2,4)1,3-thiazolo[0](2,4)1,3-thiazolino}phan (**115**) were isolated.

R_f($CHCl_3$/MeOH 5:1): 0.5

^{1}H-NMR (400 MHz, $CDCl_3$/MeOD): Mixture of diastereoisomers: 8.08; 8.04; 8.03; 7.97; 7.95; 7.92 (6 *s*, 4 H, Thiazole-CH); 6.14 – 6.03 (*m*, 4 H, NCH); 4.24 – 4.22; 3.68 – 3.64 (2 *m*, 8 H, CH_2).

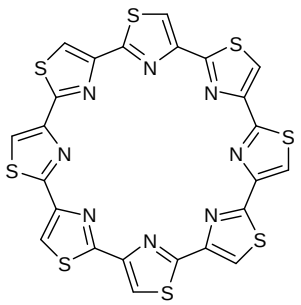


115

MS (ESI, MeOH + NaI): 664.9 (67, $[M + Na]^+$); 673.0 (5, $[M + H]^+$); 413.3 (14); 310.1 (9); 280.1 (100).

8.2.5.2.6 Optimized procedure to generate traces of **34**

11 mg of a mixture containing the tetrathiazoline **115** were dissolved in 2 ml dry DMF (solv. system) under Ar and cooled down to -5°C in an ice/brine bath. 63 µl of $BrCCl_3$ (638 µmol, 4 x 10 equiv.) were added. 30 µl of DBU (198 µmol, 4 x 3 equiv.) were diluted in 0.5 ml dry DMF and added during 10 min dropwise to the reaction. The solution was stirred under Ar for 4 h. There was no change of colour. Solvent was then removed in rv and a tiny amount of a substance with UV absorbtion at 220 and 280 nm isolated in HPLC. ESI MS showed the mass of [0.$_8$](2,4)1,3-thiazolophan (8TP, **34**).



34

MS (ESI, DMSO:MeOH 1:10 + NaI): Among a forest of other signals: 986.6 (46, [*M* + Na]$^+$); 685.5 (100), signal intensity: 2.2x10^4.

8.3 Target 3: [0.$_4$]{(2,4)1,3-Thiazolo[0](2,4)1,3-oxazolo}phan (32)

8.3.1 Investigations towards the synthesis of oxazole units

8.3.1.1 Reactions using model compounds

8.3.1.1.1 Reactions using **63**

a. Synthesis of oxazoline **72**

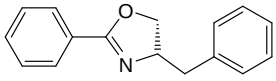
a.1 Using DAST

325 mg of **63** (1.27 mmol) were suspended in 40 ml of dry CH_2Cl_2 (*puriss.*) and cooled down to -80°C (frozen acetone) under N_2. 209 µl of DAST (1.60 mmol, 1.25 equiv.) were added and the solution was slowly let warm up to rt. Reaction was monitored on TLC (H:AcOEt 5:1). The solution was diluted with CH_2Cl_2, washed with sat. $NaHCO_3$ and brine. The aq. layers were back extracted with CH_2Cl_2, the com. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. 302 mg 2-phenyl-4-(phenylmehtyl)-1,3-oxazoline (**72**) (1.27 mmol, 100%) were isolated.

a.2 Using TsCl

255 mg of **63** (1.00 mmol) were suspended in 10 ml CH_2Cl_2 (*puriss.*) and cooled down to 0°C under N_2. 250 mg of DMAP (2.05 mmol, 2 equiv.) and 238 mg TsCl (1.25 mmol, 1.25 equiv.) were added. After three hours everything had dissolved and. The reaction was monitored on TLC (H:AcOEt 7:1). The solution was diluted with TBME (precipitation), washed twice with 0.1 M citric acid, twice with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME, the comb. org. layers dried over $MgSO_4$, filtered and dried in rv and hv. The crude product was chromatoraphically purified on SiO_2 (H:AcOEt 10:1 to 7:1). 184 mg 2-phenyl-4-(phenylmehtyl)-1,3-oxazoline (**72**) (0.775 mmol, 78%) were isolated.

R_f(H/AcOEt 5:1): 0.55



72

^{1}H-NMR (300 MHz, $CDCl_3$): 7.97 – 7.93 (m, 2 arom. H); 7.50 – 7.37 (m, 3 arom. H); 7.33 – 7.19 (m, 5 arom. H); 4.63 – 4.53 (m, 1 H, NCH); 4.35 (*dd*, ^2J = 8.4, ^3J = 8.5, 1 H, OCH_2); 4.14 (*dd*, ^2J = 8.4, ^3J = 7.4, 1 H, OCH_2); 3.24 (*dd*, ^2J = 13.7, ^3J = 5.1, 1 H, CH_2Ph); 2.73 (*dd*, ^2J = 13.7, ^3J = 8.8, 1 H, CH_2Ph).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 164.07 (*s*, 1 C, CNO); 138.12 (*s*, 1 C, arom. C); 131.41; 129.36; 128.65; 128.40; 128.37 (5 *d*, 9 C, arom. CH); 127.93 (*s*, 1 C, arom. CR); 126.60 (*d*, 1 C, arom. CH); 71.97 (*t*, 1 C, OCH_2); 68.00 (*d*, 1 C, NCH); 41.95 (*t*, 1 C, CH_2Ph).

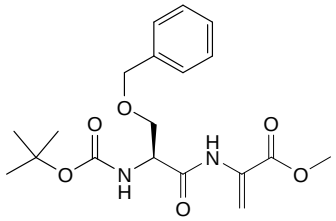
MS (ESI, MeOH): 296.1 (7); 260.1 (43, $[M + Na]^+$); 238.1 (100, $[M + H]^+$)

8.3.1.1.2 Reactions with **65**

a. Synthesis of the dehydro product **150** using TsCl

0.496 g of **65** (1.25 mmol) were dissolved in 10 ml CH_2Cl_2 (*puriss. p.a.*), 0.311 g of DMAP (2.55 mmol, 2 equiv.) and 0.257 g of TsCl (1.35 mmol, 1.05 equiv.) were added under N_2 and the solution was stirred at rt overnight. The reaction was monitored on TLC (H:AcOEt 1:1 or 5:1). The solvent was then removed by rv, the remaining oily mixture diluted with TBME, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME, the comb. org. layers dried over $MgSO_4$, filtered and dried in rv and hv. 438 mg (1.16 mmol, 93%) of Boc-Cys(SBn)-dehydroSer-OMe (**150**) were isolated.

R_f(H/AcOEt 5:1): 0.5



150

^{1}H-NMR (300 MHz, MeOD): 7.33 – 7.23 (*m*, 5 H, arom. CH); 6.44; 5.88 (2 *s*, 2 H, $=CH_2$); 4.57; 4.52 (2 *d*, ^2J = 12.1, 2 H, OCH_2Ph); 4.34 (br. *s*, 1 H, NCH); 3.80 (*s*, 3 H, OCH_3); 3.77 – 3.68 (*m*, 2 H, OCH_2); 1.45 (*s*, 9 H, $OC(CH_3)_3$).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 171.56 (*s*, 1 C, COOMe); 165.36 (*s*, 1 C, CONH); 156.31 (*s*, 1 C, Carbamate-CO); 139.14 (*s*, 1 C, *C*=CH_2); 132.89 (*s*, 1 C, arom. C); 129.42; 128.89; 128.81 (3 *d*, 5 C, arom. CH); 110.16 (*t*, 1 C, C=*C*H_2); 81.21 (*s*, 1 C, $OCMe_3$); 74.27; 70.61 (2 *t*, 2 C, Ph*C*H_2O*C*H_2); 56.74 (*d*, 1 C, NCH); 53.38 (*q*, 1 C, OCH_3); 28.67 (*q*, 3 C, OC(*C*H_3)$_3$).

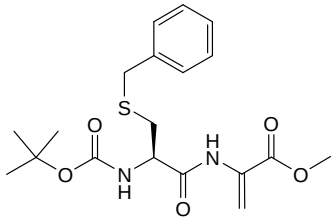
MS (ESI, CH_2Cl_2 / MeOH 1:3 + NaI): 401.3 (100, [*M* + Na]$^+$).

8.3.1.1.3 Reactions using **106**

a. Synthesis of the dehydro product **151** using TsCl

619 mg of **106** (1.50 mmol) were dissolved in 20 ml CH_2Cl_2 (*puriss. p.a.*) under N_2. 367 mg of DMAP (3.00 mmol, 2 equiv.) and 300 mg of TsCl (1.58 mmol, 1.05 equiv.) were added. The solution was stirred at rt. for 5 h. TLC (H:AcOEt 5:1) showed completion. A part of the CH_2Cl_2 was removed, the rest diluted with TBME, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with TBME, comb. org. layers dried over $MgSO_4$, filtered and dried in rv. 582 mg of Boc-Ser(OBn)-dehydroSer-OMe (**151**) (1.48 mmol, 98%) were isolated.

R_f(H/AcOEt 5:1): 0.55



151

^{1}H-NMR (300 MHz, $CDCl_3$): 8.58 (br. s, 1 H, NH); 7.34 – 7.23 (*m*, 5 H, arom. CH); 6.61 (s, 1 H, $=CH_2$); 5.92 (*d*, ^2J = 1.4, 1 H, $=CH_2$); 5.30 (br. *s*, 1 H, NH); 4.32 (br. s, 1 H, NCH); 3.80 (*s*, 3 H, OCH_3); 3.73 (*s*, 2 H, SCH_2Ph); 2.92; 2.80 (2 *dd*, ^2J = 14.1, ^3J = 6.1, 2 H, SCH_2); 1.47 (*s*, 9 H, $OC(CH_3)_3$).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 169.45 (*s*, 1 C, COOMe); 164.01 (*s*, 1 C, CONH); 155.22 (*s*, 1 C, Carbamate-CO); 137.57 (*s*, 1 C, *C*=CH_2); 130.63 (*s*, 1 C, arom. C); 128.88; 128.54; 126.95 (3 *d*, 5 C, arom. CH); 109.44 (*t*, 1 C, C=*C*H_2); 80.64 (*s*, 1 C,

$OCMe_3$); 54.49 (*d*, 1 C, NCH); 52.84 (*q*, 1 C, OCH_3); 36.41; 33.21 (2 *t*, 2 C, PhCH_2SCH_2); 28.14 (*q*, 3 C, OC(CH_3)$_3$).

8.3.1.1.4 Reactions using **64**

a. Synthesis of oxazoline **75**

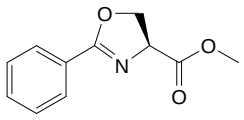
a1. Using DAST

505 mg of **64** (2.24 mmol) were dissolved under N_2 in 20 ml dry CH_2Cl_2 (*puriss. p.a.* over m.s.) and cooled down to 0°C in an ice bath. 352 µl of DAST (2.69 mmol, 1.2 equiv.) were added and the solution was slowly let warm up to rt while stirring under N_2. Reaction was monitored on TLC (H:AcOEt 1:1). After 2 h the solution was diluted with CH_2Cl_2, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with CH_2Cl_2, the comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. 464 mg of the crude product were chromatographically purified (H:AcOEt 3:1) and 443 mg of the pure methyl (4*S*)-2-penyl-1,3-oxazoline-4-carboxylate (**75**) (2.16 mmol, 96%) were isolated as a colourless oil.

a2. Using TsCl

400 mg of **64** (1.79 mmol) were dissolved in 10 ml dry CH_2Cl_2 (*puriss. p.a.* over m.s.) and cooled down to 0°C in an ice bath under N_2. 219 mg of DMAP (1.79 mmol, 1 equiv.) and 378 mg of TsCl (1.98 mmol, 1.1 equiv.) were added and the solution was stirred at 0°C, slowly warming up to rt. Reaction was monitored on TLC (H:AcOEt 1:1). After 5 h another 171 mg of TsCl (896 µmol, 0.5 equiv.) and 22 mg of DMAP (179 µmol, 0.1 equiv.) were added. When no more change could be observed on TLC, solution was diluted with CH_2Cl_2, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with CH_2Cl_2, the comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. 679 mg of the crude material were chromatographically purified (H:AcOEt 2:1 to 1:2). 128 mg of the pure methyl (4*S*)-2-penyl-1,3-oxazoline-4-carboxylate (**75**) (624 µmol, 35%, colourless oil) and 151 mg of **64** (676 µmol, 38%) were isolated.

R_f(H/AcOEt 2:1): 0.25



75

^{1}H-NMR (300 MHz, $CDCl_3$): 8.00 – 7.97 (*m*, 2 H, arom. *o*-CH); 7.50 – 7.38 (*m*, 3 H, arom. *m*-/*p*-CH); 4.96 (*dd*, ^3J = 10.6, ^3J = 7.9, 1 H, NCH); 4.70 (*dd*, ^2J = 8.7, ^3J = 7.9, 1 H, OCH_2); 4.59 (*dd*, ^2J = 8.7, ^3J = 10.6, 1 H, OCH_2); 3.82 (*s*, 3 H, OCH_3).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 171.64 (*s*, 1 C, COOMe); 166.28 (*s*, 1 C, CON); 131.87 (*d*, 1 C, arom. *p*-CH); 128.58; 128.35 (2 *d*, 4 C, arom. *o*-/*m*-CH); 126.91 (*s*, 1 C, arom. C); 69.53 (*t*, 1 C, OCH_2); 68.60 (*d*, 1 C, NCH); 52.72 (*q*, 1 C, OCH_3).

MS (ESI-MS, MeOH + NaI): 433.1 (5, [2 $M_{\mathbf{75}}$ + Na]$^+$); 228.0 (100, [*M* + Na]$^+$); 206.1 (17, [*M* + H]$^+$).

b. Synthesis of oxazole **120**

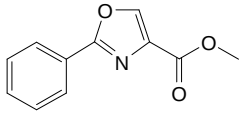
b1. Starting from oxazoline **75**

100 mg of **75** (487 µmol) were dissolved in 5 ml CH_2Cl_2 (*puriss. p.a.*)[7] and cooled down to 0°C in an ice bath. 176 µl of DBU (975 µmol, 2 equiv.) and 53 µl of $BrCCl_3$ (536 µmol, 1.1 equiv.) were added. The solution was let warm up to rt while stirring under N_2. After 16 h TLC (H:AcOEt 2:1) showed conversion to the product. Solution was diluted with AcOEt, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, comb. org. layers were dried over $MgSO_4$, filtered and the solvent was removed by rv and v. 85 mg methyl 2-penyl-1,3-oxazole-4-carboxylate (**120**) (0.42 mmol, 86%) were isolated as a colourless oil.

b2. Starting from the β-hydroxyamide **64**

40 mg of **64** (0.18 mmol) were dissolved in 2 ml DMF (solvent system)[8], cooled down to -10°C in an ice/brine bath and 35 µl of DAST (0.23 mmol, 1.5 equiv.) were added under N_2. It was let warm up to rt while stirring. After 90 min TLC (H:AcOEt 1:1) showed complete conversion to the oxazoline **75**. Solution was cooled down to 0°C again, 108 µl of DBU (713 µmol, 4 equiv.) and 27 µl of $BrCCl_3$ (0.27 mmol, 1.5 equiv.) were added. After 45 min no more **75** can be detected in TLC, quite clean conversion to methyl 2-penyl-1,3-oxazole-4-carboxylate (**120**) but a little bit of the elimination product **71** is found.

R_f(H/AcOEt 2:1): 0.5



120

^{1}H-NMR (300 MHz, $CDCl_3$): 8.30 (*s*, 1 H, Oxazole-CH); 8.13 – 8.10 (*m*, 2 H, arom. CH); 7.50 – 7.47 (*m*, 3 H, arom. CH); 3.96 (*s*, 3 H, OCH_3).

^{13}C-NMR (75.5 MHz, $CDCl_3$): 162.47; 161.74 (2 *s*, COO & CNO); 143.78 (*d*, 1 C, Oxazole-CH); 134.35 (*s*, 1 C, Oxazole-C); 131.18 (*d*, 1 C, arom. *p*-CH); 128.80 (*d*, 2 C, arom. *o*-CH); 126.84 (*d*, 2 C, arom. *m*-CH); 126.32 (*s*, 1 C, arom. C); 52.23 (*q*, 1 C, OCH_3).

MS (ESI-MS, MeOH + NaI): 429.0 (6, [2 *M* + Na]$^+$); 226.0 (100, [*M* + Na]$^+$).

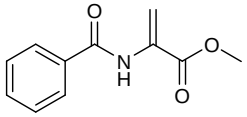
c. Synthesis of dehydro product **71**

600 mg of **64** (2.69 mmol) were dissolved in 60 ml THF (*puriss. p.a.*)[9] under N_2. 811 µl of DPPA (3.76 mmol, 1.4 equiv.) were added, the solution was stirred for 10 min and then cooled down to 0°C. 570 µl of DBU (3.76 mmol, 1.4 equiv.) were added[188]. The solution was warmed up to rt while stirring. The reaction was monitored on TLC (H:AcOEt 1:1). After 15 hours the solution was diluted with AcOEt, washed with 0.1 M citric acid, sat. $NaHCO_3$ and birne. All aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv and v. The crude product was chromatographically purified on SiO_2

[7] The reaction was also performed in DMF, pyridine and the ionic liquid 1-ethyl-3-methylimidazolium trifluoromethanesulfonate. TLC control showed comparable good conversions.
[8] Reaction was also successfully performed in CH_2Cl_2 or $CHCl_3$, in pyridine more of dehydro product **71** was found.
[9] Reaction was successfully also performed in MeCN and DMF.

(H:AcOEt 3:1). 507 mg of the pure methyl 2-benzoylaminoprop-2-enoate **71** (2.47 mmol, 79%) were isolated as colourless oil.

R_f(H/AcOEt 1:1): 0.5



71

^{1}H-NMR (300 MHz, $CDCl_3$): 8.54 (br. *s*, 1 H, NH); 7.85 – 7.83 (*m*, 2 H, 2 arom. H (*o*)); 7.55 – 7.46 (*m*, 3 H, 3 arom. H (*m*, *p*)); 6.80 (*s*, 1 H, CH_2(*cis*); 6.00 (*d*, ^2J = 0.9, 1 H, CH_2(*trans*)); 3.89 (*s*, 3 H, OMe).

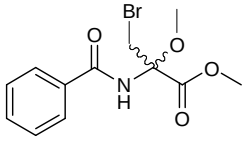
^{13}C-NMR (75.5 MHz, $CDCl_3$): 165.81 (*s*, 1 C, Ester); 164.82 (*s*, 1 C, Amide); 134.28 (*s*, 1 C, arom. C); 132.09 (*d*, 1 C, arom. *p*-CH); 131.03 (*s*, 1 C, N*C*CH_2); 128.82 (*d*, 2 C, arom. *o*-CH); 126.97 (*d*, 2 C, arom. *m*-CH_2); 108.94 (*t*, 1 C, CH_2); 53.13 (*q*, 1 C, OCH_3).

MS (+ESI-MS, MeOH + NaI): 227.9 (100, [*M*+Na]$^+$).

d. Synthesis of bromoadduct **119**

205 mg of **71** (1.00 mmol) were dissolved in 20 ml CH_2Cl_2 (*puriss. p.a.* o. m. s.). 2.5 ml MeOH (*puriss. p.a.*) and some pulverized molecular sieve A4 were added and the suspension was cooled to 0℃ in an ice bath. After 15 min 187 mg of NBS (1.05 mmol, 1.05 equiv.) were added and the suspension was slowly let warm up to rt[157]. TLC (H:AcOEt 1:1) showed after 3 h complete consumption of **71**. The suspension was then washed twice with sat. $Na_2S_2O_3$ and once with brine. The aq. layers were back extracted with CH_2Cl_2, comb. org. layers dried over $MgSO_4$, filtered and the solvent removed in rv. The product was purified by recrystallisation from H/AcOEt. 116 mg of the pure methyl 2-(benzoylamino)-3-hydroxy-2-methoxypropanoate (**119**) (367 µmol, 37%) could be isolated as colourless crystals.

R_f(H/AcOEt 1:1): 0.75; R_f(H/AcOEt 5:1): 0.14



119

^{1}H-NMR (300 MHz, $CDCl_3$): 7.88 – 7.85; 7.60 – 7.46 (2 *m*, 5 H, arom. CH); 4.71 (*d*, ^2J = 10.3, 1 H, CH_2Br); 3.94 (*s*, 3 H, OCH_3); 3.85 (*d*, ^2J = 10.3, 1 H, CH_2Br); 3.32 (*s*, 3 H, OCH_3).

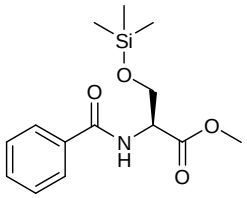
MS (ESI, MeOH + NaI): 408.3 (8); 340.1 (100, [*M* + Na]$^+$); 338.1 (90, [*M* + Na]$^+$); 258.1 (23, [*M* – HBr + Na]$^+$); 236.1 (17, [*M* – HBr + H]$^+$); 222.1 (8, [*M* – HBr – CH_2 + H]$^+$); 204.1 (16, [*M* – HBr – MeOH + H]$^+$); 105.1 (10).

e. Protection of β-hydroxyamides with trialkylsilyl groups

e1. Synthesis of trimehtylsilanol **152**

100 mg of **64** (448 µmol) were dissolved in 3 ml CH_2Cl_2 (*puriss. p.a.*) and cooled down to 0℃ in an ice bath. 0.1 ml of TMSCl (0.8 mm ol, 1.8 equiv.) and 160 µl of Et_3N (1.15 mmol, 2.5 equiv.) were added. The solution was let warm up to rt while stirring under N_2 overnight. The solution was then diluted with AcOEt, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with AcOEt and the comb. org. layers dried over $MgSO_4$. The salt was removed by filtration and the solvent by rv. 116 mg of methyl (2*S*)-2-(benzoylamino)-3-(trimethylsilyloxy)propanoate (**152**) were isolated (392 µmol, 88%).

^{1}H-NMR (300 MHz, $CDCl_3$): 7.95 – 7.93; 7.66 – 7.54 (2 *m*, 5 H, arom. CH); 7.09 (br. *d*, ^3J = 7.8, 1 H, NH); 4.98 (*dt*, ^3J = 8.1, ^3J = 2.9, 1 H, NCH); 4.24 (*dd*, ^2J = 10.5, ^3J = 2.7, 1 H, CH_2); 4.05 (*dd*, ^2J = 10.5, ^3J = 3.2, 1 H, CH_2); 3.90 (*s*, 3 H, OCH_3); 0.20 (*s*, 9 H, $OSi(CH_3)_3$).



152

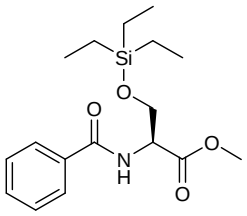
MS (ESI, MeOH + NaI): 360.4 (6); 318.2 (100, $[M + Na]^+$); 246.1 (9, $[M – SiMe_3 + H + Na]^+$).

e2. Synthesis of the triethylsilanol **132**

204 mg of **64** (914 µmol) were dissolved in 16 ml dry pyridine, 0.75 ml of TES-Cl (4.5 mmol, 5 equiv.) were added and the solution was heated up to 60°C for 1 h [239]. The solvent was then removed under reduced pressure, freezing it out in a cooling trap. The remaining oil was diluted in AcOEt, washed twice with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, comb. org. layers were dried over $MgSO_4$, filtered and solvent removed in rv. The crude product was chromatographically purified on SiO_2 (hexane, then H:AcOEt 2:1). 275 mg methyl (2*S*)-2-(benzoylamino)-3-(triethylsilyloxy)propanoate (**132**) (815 µmol, 89%) were isolated as a colourless oil.

R_f(H/AcOEt 1:1): 0.57; R_f(H/AcOEt 2:1): 0.28

^{1}H-NMR (300 MHz, $CDCl_3$): 7.84 – 7.81 (*m*, 2 H, arom. *o*-CH); 7.53 – 7.43 (*m*, 3 H, arom. *m*/*p*-CH); 7.00 (br. *d*, ^3J = 7.8, 1 H, NH); 4.88 (*dt*, ^3J = 8.3, ^3J = 2.7, 1 H, NCH); 4.17 (*dd*, ^2J = 10.1, ^3J = 2.7, 1 H, OCH_2); 3.97 (*dd*, ^2J = 10.1, ^3J = 3.1, 1 H, OCH_2); 3.79 (*s*, 3 H, OCH_3); 0.93 (*t*, ^3J = 7.9, 9 H, 3 TES-CH_3); 0.58 (*q*, ^3J = 7.9, 6 H, 3 TES-CH_2).



132

f. Conversion of silanol **132** into oxazoline **75**

40 mg of **132** (0.12 mmol) were dissolved in 1 ml CH_2CH_2 (techn.) at rt under N_2. 15 µl of DAST (0.11 mmol, 1 equiv.) were added[231, 232]. According to TLC (H:AcOEt 2:1) 50% were converted to **75** after 5 h, reaction overnight gave clean and quantitative conversion to **75**. Product was not isolated.

g. Conversion of silanol **132** into oxazole **120**

40 mg of **132** (0.12 mmol) were dissolved in 1 ml CH_2CH_2[10] (techn.) at rt under N_2. 15 µl of DAST (0.11 mmol, 1 equiv.) were added. After 20 min reaction to the oxazoline **75** was completed according to TLC (H:AcOEt 2:1). Solution was cooled down to 0°C, 54 µl of DBU (0.36 mmol, 3 equiv.) and 12 µl of $BrCCl_3$ (0.12 mmol, 1 equiv.) were added. Another 30 min later clean and qunatitative conversion to **120** is completed. Product was not isolated.

8.3.1.1.5 Reactions using **116**

a. Synthesis of **127** by debenzylation and tosylation

269 mg of **116** (840 µmol) were dissolved in 20 ml CH_2Cl_2 (*puriss. p.a.*) and 1.4 g of Ts_2O (4.3 mmol, 5 equiv.) and 1.2 ml of $BF_3.Et_2O$ (~7 M, 8.4 mmol, 10 equiv.) were

[10] Reaction is also possible in $CHCl_3$ but is less clean. Under the addition of K_2CO_3 in granular or powdered form at 0°C formation of little dehydro pr oduct could be observed.

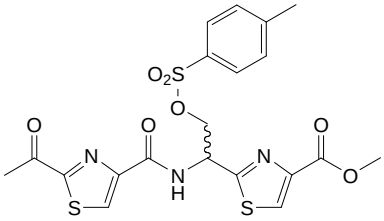
added and the solution was stirred under N_2 overnight at 50°C. The conversion was monitored on TLC (H:AcOEt 1:1). The solution was then diluted with AcOEt, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. The crude product was adsorbed to SiO_2 and chromatographically purified on SiO_2 (H:AcOEt 3:1 to 1:1). 122 mg of a mixture of products, containing also **127**, the elimination product **129** and the alcohol **128**, could be isolated.

8.3.1.1.6 Reactions using **99**

a. Debenzylation and tosylation

100 mg of **99** (224 µmol) were dissolved in 4 ml CH_2Cl_2 (*puriss*. *p.a.*) at rt under N_2. 322 µl of $BF_3.Et_2O$ (2.20 mmol, 10 equiv.) and 740 mg of Ts_2O (2.30 mmol, 10 equiv.) were added. After 6 h stirring at rt almost no conversion could be observed by TLC (H:AcOEt 1:1). The solution was heated up to reflux for 20 h, then diluted with AcOEt, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with AcOEt, the comb. org. layers were dried over $MgSO_4$, filtered and the solvent removed in rv. The crude material was adsorbed at SiO_2 and the mixture of products chromatographically purified on SiO_2. 18 mg of the quite pure methyl 2-{1-[(2-acetyl-1,3-thiazole-4-yl)carbonylamino]-2-(tosyloxy)ethyl}-1,3-thiazole-4-carboxylate (**130**) (35 µmol, 16%) and 25 mg of the almost clean alcohol **104** (70 µmol, 31%) were isolated. An unquantified amount oxazoline **118** was also found.

R_f(H/AcOEt 1:3): 0.5



130

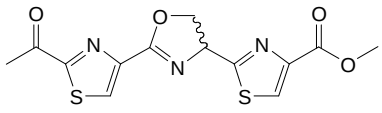
^{1}H-NMR (300 MHz, $CDCl_3$): 8.44 (*s*, 1 H, Thiazole-CH); 8.27 (br. *d*, ^3J = 8.2, 1 H, NH); 8.14 (*s*, 1 H, Thiazole-CH); 7.72 (*d*, ^3J = 7.9, 2 H, Ts-*o*-CH); 7.30 (*d*, ^3J = 7.9, 2 H, Ts-*m*-CH); 5.78 (*dt*, ^3J = 8.2, ^3J = 4.8, 1 H, NCH); 4.72 (*dd*, ^2J = 10.5, ^3J = 5.6, 1 H, OCH_2); 4.58 (*dd*, ^2J = 10.5, ^3J = 4.5, 1 H, OCH_2); 3.95 (*s*, 3 H, OCH_3); 2.79 (*s*, 3 H, $(CO)CH_3$); 2.43 (*s*, 3 H, Ts-CH_3).

MS (ESI, MeOH + NaI): 532.0 (100, $[M + Na]^+$); 510.0 (12, $[M + H]^+$); 425.4 (5); 406.1 (6); 378.1 (27, $[M – SO_2C_7H_7 + H + Na]^+ = [M_{\mathbf{104}} + Na]^+$); 360.4 (6, $[M – C_7H_7SO_3H + H + Na]^+ = [M_{\mathbf{118\ /\ 117}} + Na]^+$); 356.1 (11, $[M – SO_2C_7H_7 + 2\ H]^+ = [M_{\mathbf{104}} + H]^+$); 338.1 (61, $[M – C_7H_7SO_3H + 2\ H]^+ = [M_{\mathbf{118\ /\ 117}} + H]^+$).

b. Synthesis of oxazoline **118** starting with β-hydroxyamide **104**

15 mg of **104** (42 µmol) were suspended in 4 ml CH_2Cl_2 (*puriss. p.a.* o.m.s.). 6 mg of DMAP (49 µmol, 1.2 equiv.) and 18 mg of TsCl (94 µmol, 2.2 equiv.) were added. The suspension was stirred at rt under N_2 overnight. TLC (H:AcOEt 1:3) showed around 50% conversion. Another 10 mg of TsCl (52 µmol, 1.2 equiv.) and 6 mg of DMAP (49 µmol, 1.2 equiv.) were added and the solution was heated to 40°C for 20 h. The suspension was then diluted with AcOEt, washed once with 0.1 M citric acid, once with sat. $NaHCO_3$ and once with brine. All aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. The crude material was chromatographically purified on SiO_2 (H:AcOEt 1:1). 5 mg of the pure methyl 2-[2-(2-acetyl-1,3-thiazol-4-yl)-1,3-oxazolin-4-yl]-1,3-thiazole-4-carboxylate **118** (15 µmol, 35%) were isolated.

MS (ESI, MeOH + NaI): 576.1 (16); 554.2 (18); 532.0 (100, $[M + TsOH + Na]^+ = [M_{130} + Na]^+$); 510.1 (10, $[M + TsOH + H]^+ = [M_{130} + H]^+$); 486.1 (8); 468.1 (11); 446.2 (9); 378.1 (31, $[M + H_2O + Na]^+ = [M_{104} + Na]^+$); 360.1 (100, $[M + Na]^+$); 338.2 (47, $[M + H]^+$).



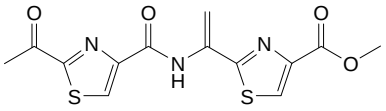
118

c. Synthesis of dehydro product **117**

60 mg **99** (0.14 mmol) were dissolved in 3 ml CH_2Cl_2 (*puriss. p.a.*), 300 µl $(CF_3CO)_2O$ (10 vol%) and 192 µl $BF_3.Et_2O$ (1.3 mmol, 10 equiv.) were added and the solution was stirred at rt under N_2 overnight. TLC (H:AcOEt 1:1) did not show complete conversion, so another 150 µl $(CF_3CO)_2O$ (5 vol%) and 96 µl $BF_3.Et_2O$ (0.7 mmol, 5 equiv.) were added. The solution was stirred for another 20 h, then cooled down to 0°C and 1 ml of a 1 M solution of DBU in DM F (1 mmol) was added. 1 h later diluted in AcOEt, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. Aq. layers back extracted, org. layers dried over $MgSO_4$, filtered and solvent removed in rv. The crude product was chromatographically purified on SiO_2. 5 mg methyl 2-{1-[(2-acetyl-1,3-thiazol-4-yl)-carbonylamino]ethen-1-yl}-1,3-thiazole-4-carboxylate (**117**) (15 µmol, 11%) were isolated.

R_f(H/AcOEt 1:1): 0.65

^{1}H-NMR (300 MHz, $CDCl_3$): 10.52 (br. *s*, 1 H, NH); 8.48; 8.17 (2 *s*, 2 H, Thiazole-CH); 6.67; 5.62 (2 *d*, ^2J = 1.9, 2 H, $=CH_2$); 3.96 (*s*, 3 H, OCH_3); 2.89 (*s*, 3 H, $(CO)CH_3$).



117

MS (ESI, MeOH + NaI): 411.2 (17); 360.1 (100, $[M + Na]^+$); 338.1 (5, $[M + H]^+$).

8.3.1.2 Reactions on the macrocycle

8.3.1.2.1 Most promising procedure for the formation of tetrachloride **74**

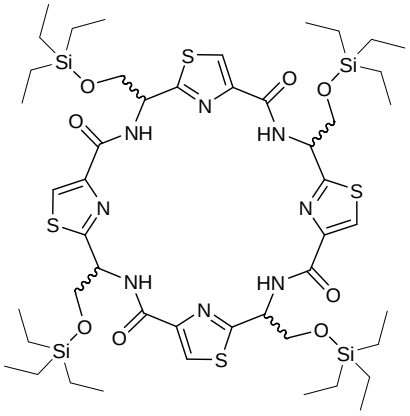
108 mg of LiCl (previously dried at 100°C in hv during one hour) were dissolved in 5 ml dry DMF (solv. system) under Ar. 40 mg of **59** (59 mmol) were added and the solution was cooled down to 0°C in an ice bath. 42 ml of DAST (320 mmol, 4 x 1.5 equiv.) were added and the solution was let warm up to rt while stirring. The progress of the reaction was monitored on TLC ($CHCl_3$:MeOH 5:1). The solvent was partially removed in rv, the remaining solution diluted with AcOEt, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and solvent removed in rv. The 29 mg of crude material contained some of the product 3,11,19,27-tetra(chloromehtyl)-1,9,17,25-tetroxo-2,10,18,26-tetraza[3.$_4$](2,4)1,3-thiazolophan (**74**).

8.3.1.2.2 Synthesis of tetrasilanole **133**

50 mg of **59** (73 µmol) were dissolved in 4 ml dry pyridine (*puriss. p.a.* o.m.s.). 0.2 ml of TES-Cl (1.2 mmol, 4 x 4 equiv.) were added. The solution was heated up to 60°C under N_2 for two hours and the conversion was monitored on TLC (H:AcOEt 1:4). The solution was then diluted with AcOEt, washed with 0.1 M citric acid, sat. $NaHCO_3$ and brine. All aq. layers were back extracted with AcOEt, comb. org. layers dried over $MgSO_4$, filtered and the solvent removed in rv. The crude material was chromatographically purified on SiO_2. 43 mg of the pure 3,11,19,27-

tetra[(triethylsilyloxy)mehtyl]-1,9,17,25-tetroxo-2,10,18,26-tetraza[3.4](2,4)1,3-thiazolophan (**133**) (37 µmol, 50%) were isolated as a solidifying oil.

R_f(H/AcOEt 1:5): 0.62



133

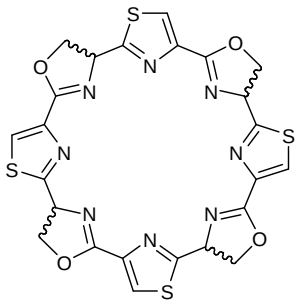
^{1}H-NMR (300 MHz, $CDCl_3$): mixture of isomers: 8.47 – 8.25 (*m*, 4 H, NH); 8.17; 8.14; 8.14 (3 *s*, 4 H, Thiazole-CH); 5.77 – 5.36 (*m*, 4 H, NCH); 4.55 – 3.69 (*m*, 8 H, OCH_2); 1.03 – 0.73 (*m*, 36 H, Silyl-CH_3); 0.65 – 0.40 (*m*, 24 H, Silyl-CH_2).

MS (ESI, MeOH + NaI): 1309.4 (6); 1159.6 (100, [*M* + Na]$^+$); 1027.5 (5, [*M* – Et_3SiOH + Na]$^+$).

8.3.1.2.3 Synthesis of tetraoxazoline **61**

20 mg of **133** (18 µmol) were dissolved in 1.2 ml dry CH_2Cl_2 (*puriss. p.a.*). 33.3 mg of XtalFluor-E (144 µmol, 8 equiv.) were added at rt under Ar and stirred for 72 h. The progress of the reaction was monitored on TLC (CH_2Cl_2:MeOH 20:1). The mixture was then treated with 1.0 ml MeOH (*puriss. p.a.*) and stirred for another 10 min at rt under Ar. The crude product was adsorbed onto previously dried neutral Alox and chromatographically purified over neutral dry Alox (CH_2Cl_2:MeOH 99:1, both *purum*). 4.5 mg (7.4 µmol, 41%) of a mixture of diastereoisomers of [0.4]{(2,4)1,3-thiazolo[0](2,4)1,3-oxazolino}phan (DH4TOP, **61**) was isolated.

R_f(CH_2Cl_2/MeOH 20:1): 0.49



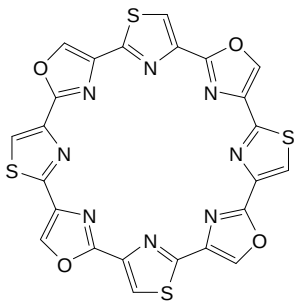
61

^{1}H-NMR (300 MHz, $CDCl_3$): mixture of diastereoisomers: 7.97; 7.93; 7.90; 7.86; 7.82; 7.76 (6 *s*, 4 H, thiazole-CH); 5.60 – 5.48 (*m*, 4 H, NCH); 4.91 – 4.85; 4.73 – 4.64 (2 *m*, 8 H, CH_2).

MS (ESI, MeOH/DMSO 10:1 + NaI): 780.9 (25, [*M* + TES-OH + H_2O + Na]$^+$); 682.2 (7); 649.0 (18); 631.0 (100, [*M* + Na]$^+$); 609.1 (20, [*M* + H]$^+$).

8.3.1.2.4 Formation of 0.4TOP (**32**)

4.5 mg **61** (7.4 µmol) were dissolved in 2.5 ml dry DMF (solvent system, c = 3 mM) and 8.9 µl $BrCCl_3$ (90 µmol, 12 equiv.) were added. A first precipitate was observed. The suspension was cooled down to 0°C and 5.3 ml of a 50 mM solution of DBU (270 µmol, 36 equiv.) in DMF were added over 10 min. The suspension was stirred at rt under N_2 for 16 h. The suspension was then diluted with 25 ml H_2O, the precipitate was collected by centrifugation, dissolved in DMSO and again precipitated by addition of H_2O. The colourless solid was collected by centrifugation. The isolated material contained [0.4]{(2,4)1,3-thiazolo[0](2,4)1,3-oxazolo}phan (4TOP, **32**) according to MALDI-MS and ^{1}H-NMR.



32

^{1}H-NMR (400 MHz, DMSO): 9.17 (*s*, 4 H, thiazole-CH); 8.56 (*s*, 4 H, oxazole-CH).

MS (MALDI, Matrix PAC HCCA): 834.0 (6); 706.0 (100, $[M + CH_2(C_6H_4O)]^+$); 674.1 (17); 660.0 (21); 638.9 (12, $[M + Na]^+$); 622.9 (74, $[M + Na]^+$); 618.0 (15, $[M + H_2O]^+$); 601.0 (18, $[M + H]^+$).

Part III

^{18}O Isotopic Labeling of Amino Acids

9. Introduction

Much of our synthetic efforts towards new telomestatin analogs utilize standard peptide chemistry. As coupling reagents, the benzotriazole-based TBTU and PyBOP (*Figure 9.1*) were used.

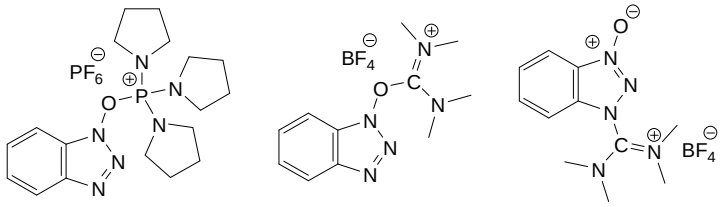


Figure 9.1: *Coupling reagents PyBOP and TBTU*

These reagents are widely used, but their precise mechanism of action is still a matter of debate[240-243]. Two pathways for the initiation of the coupling reaction are theoretically possible. The acid is either (A) attacking the phosphorous or the uronium-carbon or (B) attacking the triazole-nitrogen. These possible mechanisms are presented in *Scheme 9.1* with PyBOP as the coupling reagent. While much research has been aimed at elucidating the nature of the reactive species[242, 244, 245], the question of the first intermediate formed during amino acid activation has not previously been addressed. Specifying the carboxylic acid oxygens versus the PyBOP oxygen by using a doubly ^{18}O isotopic labeled amino acids should prove a different distribution of ^{18}O in the products for path A or B (*Scheme 9.1*).

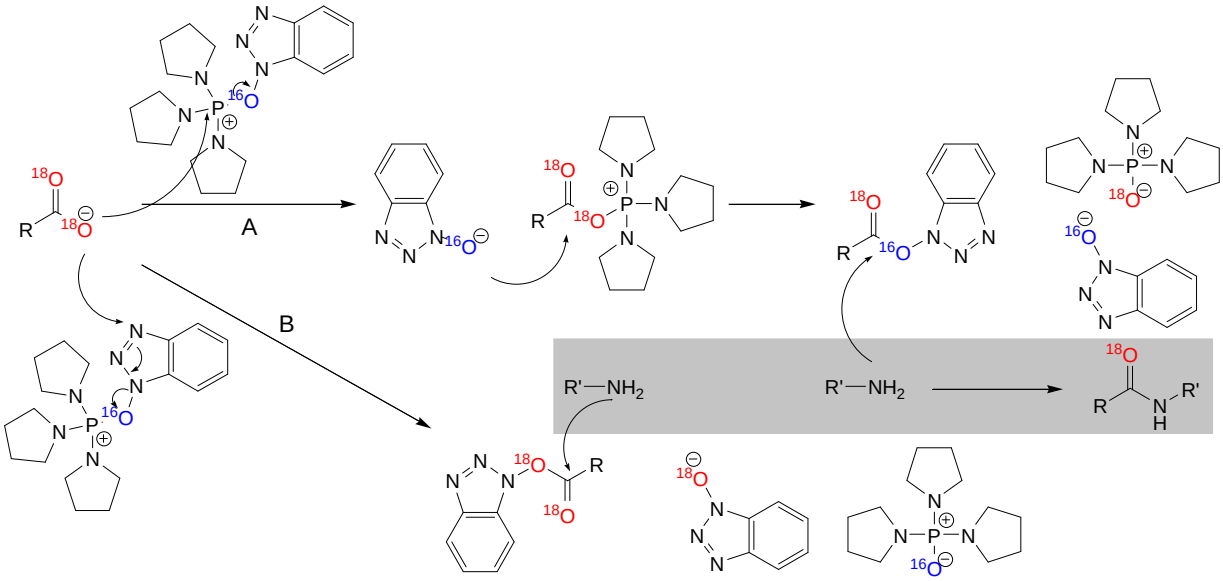
Isotopically labeled amino acids have found wide application in structure elucidation of peptides and proteins. In addition to ^{17}O NMR studies[246-254], 2D-IR techniques are emerging as important new tools for probing protein folding and ligand binding[255-267]. Due to its time resolution in the subpicosecond range, time resolved 2D-IR spectroscopy can report the rapid evolution of non-equilibrium conformational states to reveal folding pathways[256-267]. These techniques rely upon site-specific incorporation of ^{18}O-labeled amino acids into peptides and proteins to serve as spectroscopic probes[260-267]. To date, only aliphatic, aromatic, and sulfur-

from 20 – 85% for products containing a single ^{17}O atom[251, 252]. In order to expand the range of amino acids labeling, we developed a new methodology using mild conditions compatible with different protective groups that provide efficiency for double isotopically labeled acids[273]1.

[1] ^{18}O labeling and PyBOP reactivity experiments were conducted with Birgit Lauber during her project practicum in our lab.

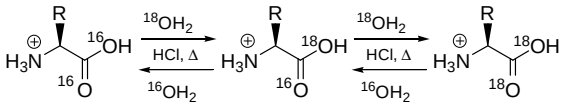
containing amino acids have been utilized in such studies because currently used methods for preparing ^{18}O-labeled amino acids result in uniform ^{18}O-labeling of side chain and α-carboxylic acids[268-272].

Scheme 9.1. *Two possible mechanisms for PyPOP-mediated peptide bond formation*



^{18}O-labeling is normally conducted by heating the carboxylic acid with $^{18}OH_2$ in the presence of a strong acid to catalyze exchange (*Scheme 9.2*). By using an excess of ^{18}O water, the reported yields for isotopic enrichments are typically 75 – 85%[255, 268-272]. A labeling procedure for Fmoc-protected amino acids using multiple rounds of acid-catalyzed $^{18}OH_2$ equilibration recently reported ^{18}O enrichments of 90 – 96% for Phe, Ala, Gly, and Phe[255]. This method can be very efficient with respect to $^{18}OH_2$ consumption, but the protective groups typically used for functionalized side chains (Boc, Trt, *t*Bu, etc.) are not stable under the strongly acidic conditions used to catalyze exchange. These conditions can therefore cause uniform labeling of Asp, Glu, Asn and Gln residues[270-272].

Scheme 9.2. *Typical equilibrium-based ^{18}O labeling reaction*

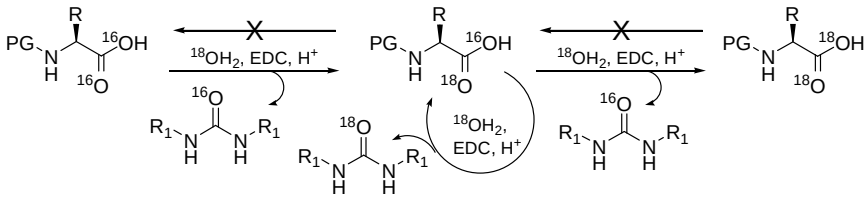


Single-turnover $^{17}OH_2$ saponification reactions of pentafluorophenyl esters have recently been reported[251, 252]. These reactions tolerate acid labile (*t*Bu) protective groups and can selectively label α-carboxylic acids with isotopic enrichments ranging

10. Results and Discussion

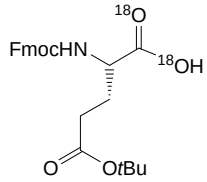
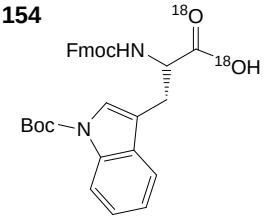
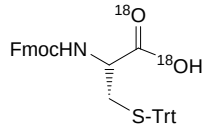
We report a mild and selective reaction for the isotopic labeling of amino acids, peptides and proteins to provide highly enriched materials for NMR, IR, and MS studies. Making use of a non-equilibrium multiple-turnover reaction, the carboxylic acid is reacted with a carbodiimide to form an *O*-acylisourea that is hydrolysed by ^{18}O water. After one round of activation and hydrolysis, the mixed ^{16}O/^{18}O carboxylic acid will be re-activated and hydrolysed again and again (*Scheme 10.1*). Since carbodiimides react more rapidly with carboxylates than with water[274], one-pot reactions containing an excess of coupling reagent and $^{18}OH_2$ result in multiple rounds of *O*-acylisourea formation and hydrolysis to provide isotopic enrichments of approximately 92 – 95 % for both oxygen atoms of the carboxylic acid (*Table 10.1*).

Scheme 10.1. *Non-equilibrium, multiple turnover ^{18}O labeling reaction*



While heavy atom isotope effects are typically considered negligible for preparative-scale reactions, a modest kinetic bias should favor formation of the double–labeled ^{18}O product. The ^{16}O atom in the mixed carboxylic acid (*Scheme 10.1*) should reacts slightly faster than ^{18}O to form the corresponding ^{16}*O*-acylisourea[275]. The resulting effect on efficiency depends on the kinetic isotope effect (KIE) to the exponential power of reaction turnovers (n), i.e. $(KIE)^n$. In theory, a very small KIA of 1.010 would result in cumulative KIA's of about 11% and 270% after 10 and 100 turnovers, respectively. This should serve to decrease $^{18}OH_2$ consumption, especially for large-scale reactions. In the current set of examples, only six turnovers/equivalents of $^{18}OH_2$ (if 100 % enriched) are theoretically sufficient for making amino acids with 98% isotopic enrichment, even without taking kinetic isotope effects into account.

Table 10.1. *^{18}O enrichment and isolated yield*

entry	product	% enrichment *	isolated yields
153		93%	94%
154		92%	88%
155		95%	95%

Our protocol for the selective labeling of α-carboxyl groups of amino acids utilizes commercially available, protected amino acids and coupling reagents. All starting materials were dried before use. To activate the carbodiimide and suppress racemization of the *O*-acylisourea intermediate, an excess of 3,5-dimethylpyridinium bromide (pK_a ~ 5) is included as a dry proton source. We conducted isotope labeling experiments with three different Fmoc-protected amino acids containing a variety of acid-labile protective groups (*Table 10.1*). In a typical reaction, 50 equiv. of $^{18}OH_2$ (95% ^{18}O), 30 equiv. of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and 20 equiv. of 3,5-dimethylpyridinium bromide in dry DMF were reacted overnight at room temperature. Reactions containing 18 equiv. of ^{18}O and 10 equiv. of carbodiimide furnished products with only 55% enrichment. While other carbodiimides (DCC, DIC, etc.) worked well for this reaction, the water solubility of EDC greatly simplified purification as the resulting ^{16}O and ^{18}O-containing ureas were washed away with water. Isolated yields for **153** and **155** were nearly quantitative (94 – 95%), while the yield for Fmoc-Trp(Boc)-^{18}OH (**154**) was slightly lower (88%) due to the extreme acid sensitivity of the Boc-protected indole (*Table 10.1*). Each product **153** – **155** was characterized by MS, IR, NMR, and polarimetry. Selective ^{18}O labeling of the α-carboxylic acids was observed by ^{13}C-NMR by using mixtures of

starting materials and isolated products. Consistent with previous reports of other acids[268, 276], the ^{18}O-labeled α-carbon of products **153** – **155** were shifted upfield by ~ 0.05 ppm as compared to ^{16}O-containing starting materials. Selective labeling was also indicated by mass spectrometry as exactly two ^{18}O atoms were incorporated into each product. Quantitative ESI mass spectrometry of **153** – **155** revealed total isotopic enrichments of 92 – 95% (*Table 10.1*). This enrichment is very similar to the purity of the $^{18}OH_2$ (95% ^{18}O) added to each reaction. Based on these results, we estimate that five or more cycles of activation and hydrolysis occurred during each reaction. Similar results were also obtained for relatively simple amino acids like Fmoc-Gly-^{18}OH. To the best of our knowledge, these are the first reported examples of kinetically-enhanced multiple turnover reactions used for preparative isotopic labeling.

Together with Birgit Lauber, preliminary experiments for tracking ^{18}O during PyBOP mediated amide bond formation were performed. The labelled amino acids were reacted in dry DMF with benzylamine using PyBOP and NMM. The mixture was then fractionated and the tripyrrolidinephosphinoxide was analysed with ESI-MS. Different ratios of labelled to unlabeled phosphinoxides were observed, ranging from 2:1 to 1:2. This result can not be reconciled with a single mechanism (*Scheme 9.1*). It is not currently known whether unrelated oxygen exchange reactions can take place under these reaction conditions.

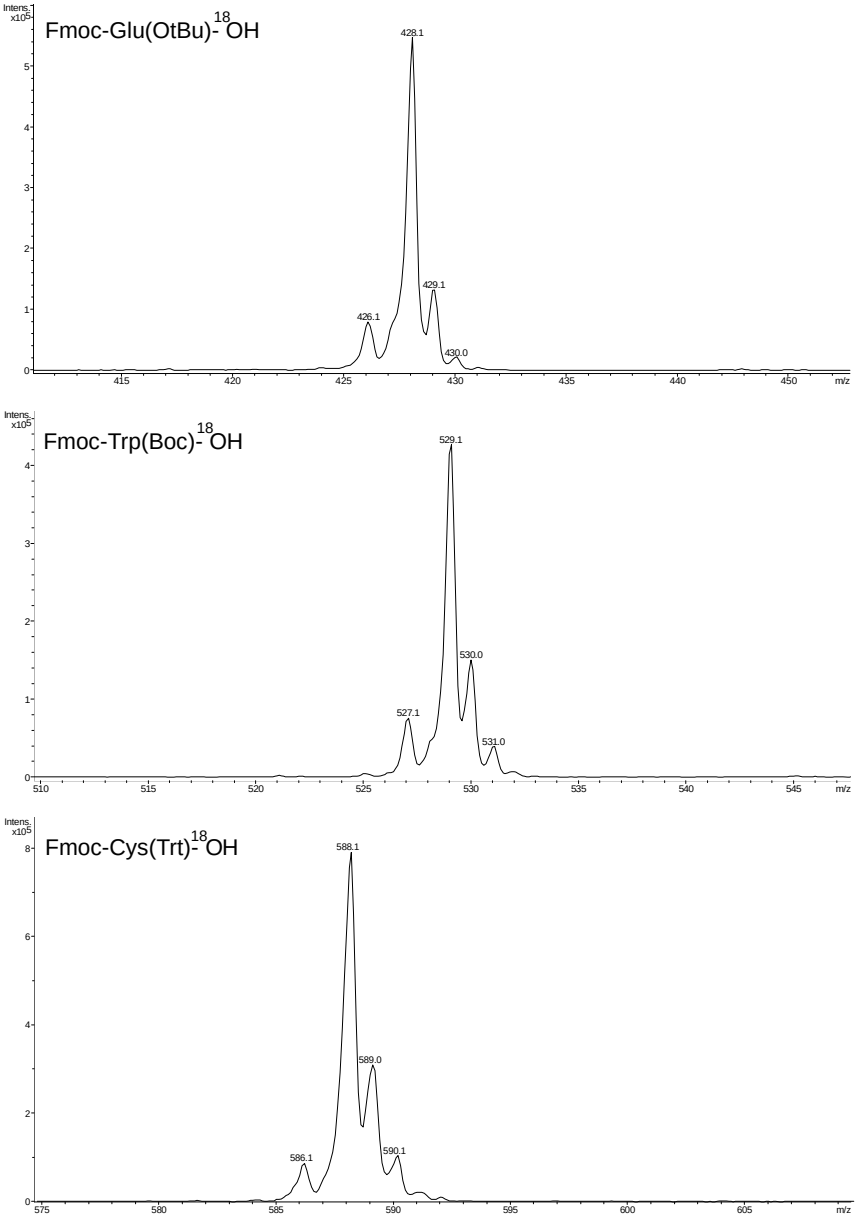


Figure 10.1: *ESI mass spectra of compounds **153** – **155**. Total % isotopic enrichments were calculated by taking the peak intensities of the $^{16}O/^{16}O$ species as 0 %, the $^{16}O/^{18}O$ species as 50 %, and the $^{18}O/^{18}O$ species as 100 % enrichment.*

11. Synthetic Procedures

11.1 Materials and methods

^{18}O-labeled water was purchased as a normalized 95% ^{18}O solution (5% ^{16}O) from Armar Chemicals (Döttingen, Switzerland). 3,5-dimethylpyridine was purchased from Aldrich in *purum* quality, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) was purchased in *purum* quality from Fluka. DMF purification grade from Aldrich was dried over a column of 5 Å molecular sieves in a Pure Solvent System from Innovative Technology (Newberyport, MA). All amino acids were obtained from Bachem AG.

Immediately before ^{18}O labelling all reagents and starting materials were extensively dried by making sonicated suspensions in dry CH_3CN and removing the residual water as a CH_3CN azeotrope under reduced pressure. This procedure was repeated three times prior to labeling. Oven-dried glassware and stirrer bars were used and all reactions and starting materials were kept under dry N_2.

For preparative HPLC a *VARIAN* Prep Star Model 218 equipped with a *Waters* X Bridge Column (19 x 50 mm, 5 µm C18 particles) was used. ^{1}H- and ^{13}C-NMR spectra were measured on *Bruker ARX-300* or *AV-400* spectrometers (*Bruker,* Karlsruhe, Germany). The chemical shift values are given in ppm relative to the residual signal from TMS (δ = 0.00) for ^{1}H-NMR and CD_3OD (δ = 49.00 ppm) for ^{13}C-spectra. The coupling constants *J* are given in Hz; resonance multiplicity is described as *s* for singlet, *d* for doublet, *dd* for doublet of doublet, *t* for triplet, *m* for multiplet; and *br* is used to indicate broad signals. All data processing was carried out with Topspin and Xeasy (*Bruker*). IR spectra were collected using a JASCO FT/IR-4100 spectrometer equipped with an anvil sample compressor for matrix-free data collection. $[\alpha]_D$-values were measured on a *Perkin-Elmer* Polarimeter 241 using solutions of 10 – 20 mM in the indicated solvent.

Mass spectra were measured by the Organic Chemistry Institute of the University of Zürich. ESI mass spectra were performed on a *Bruker ESQUIRE-LC* quadrupole ion trap instrument (*Bruker Daltonik GmbH,* Bremen, Germany), equipped with a combined Hewlett-Packard Atmospheric Pressure Ion (API) source (*Hewlett-Packard Co.*, Palo Alto, CA, USA). Ions with more than 5% intensity are given.

11.2 Synthesis of Fmoc-L-Glu(O*t*Bu)-^{18}OH (**153**)

850 mg dry 3,5-dimethylpyridine hydrobromide (4.52 mmol, 20 equiv.) were suspended in 2 ml dry DMF under N_2, 430 mg dry EDC.HCl (2.24 mmol, 10 equiv.) and 225 µl $^{18}OH_2$ (95%, 11.3 mmol, 50 equiv.) were added. 100 mg dried Fmoc-L-Glu(O*t*Bu)-OH.H_2O (225 µmol), dissolved in 3 ml dry DMF, was immediately added by syringe. The mixture was stirred at rt for 18 h under N_2. A second portion dry EDC.HCl (430 mg, 2.24 mmol, 10 equiv.) was added and the mixture was stirred for another 8 h at rt. A third portion dry EDC.HCl (430 mg, 2.24 mmol, 10 equiv.) was added and stirred for another 15 hours. The reaction was diluted into AcOEt (30 ml), washed three times with 0.1 M citric acid (25 ml), once with 0.1 M citric acid in brine (25 ml). All aq. layers were back extracted once with one portion AcOEt (30 ml). Comb. org. layers were dried over $MgSO_4$, filtered, and the solvent removed by rv and hv. The isolated material was further purified by preparative HPLC using an CH_3CN/H_2O gradient (flow rate of 6 ml/min) of 60% to 70% CH_3CN (0.05% TFA) over 10 minutes to yield 90.8 mg (212 mmol, 94%) of a colourless solid.

Analytical data for Fmoc-L-Glu(O*t*Bu)-^{16}OH.H_2O (starting material):

$[\alpha]_D^{20}$ (*MeOH*): - 9.06
IR (neat): 1720.2*s*, 1527.4*w*; 1447.3*w*; 1371.1*w*; 1331.6*w*; 1248.7*m*; 1155.2*m*; 1054.9*w*; 947.8*w*; 844.7*w*; 747.3*w*.

^{1}H-NMR (300 MHz, MeOD): 7.77 (*d*, ^3J = 7.3, 2 H, Fluorenyl-C$^{4'/5'}$-H); 7.68 – 7.64 (*m*, 2 H, Fluorenyl-C$^{1'/8'}$-H); 7.39 – 7.27 (*m*, 4 H, Fluorenyl-C$^{2'/3'/6'/7'}$-H); 4.35 – 4.32 (*m*, 2 H, C$^{10'}$-H); 4.24 – 4.09 (*m*, 2 H, C^2-H & C$^{9'}$-H); 2.34 (*t*, ^3J = 7.2, 2 H, C^4-H); 2.21 – 2.10; 1.95 – 1.83 (2 *m*, 2 H, C^3-H); 1.44 (*s*, 9 H, C$(CH_3)_3$).

^{13}C-NMR (*75.5 MHz, MeOD*): 175.39 (*s*, 1 C, C^{1}OOH); 173.84 (*s*, 1 C, C^{5}OO*t*Bu); 158.65 (*s*, 1 C, OCONH); 145.31; 145.15; 142.56 (3 *s*, 4 arom. C); 128.78; 128.16; 126.27; 120.92 (4 *d*, 8 arom. CH); 81.83 (*s*, 1 C, OCMe$_3$); 67.99 (*t*, 1 C, OCH$_2$); 54.53 (*d*, 1 C, NHCH); 48.37 (*s*, 1 C, OCH$_2$*C*H); 32.70 (*t*, 1 C, C^{3}H$_2$); 28.35 (*q*, 3 C, OC(*C*H$_3$)$_3$); 27.98 (*t*, 1 C, C^{4}H$_2$).

Analytical data for Fmoc-L-Glu(O*t*Bu)-^{18}OH (product):

$[\alpha]_D^{20}$ (*MeOH*): - 9.28
IR (neat): 1707.7*s*; 1527.4*w*; 1447.*3w*; 1372.1*w*; 1331.6*w*; 1251.6*m*; 1155.2*m*; 1053.9*w*; 746.3*m*.

^{1}H-NMR (400 MHz, MeOD): 7.79 (*d*, ^3J = 7.5, 2 H, Fluorenyl-C$^{4'/5'}$-H); 7.69 – 7.65 (*m*, 2 H, Fluorenyl-C$^{1'/8'}$-H); 7.40 – 7.29 (*m*, 4 H, Fluorenyl-C$^{2'/3'/6'/7'}$-H); 4.35 (*d*, 2 H, ^3J = 7.3, C$^{10'}$-H); 4.24 – 4.19 (*m*, 2 H, C^2-H & C$^{9'}$-H); 2.34 (*t*, ^3J = 7.3, 2 H, C^4-H); 2.20 – 2.11; 1.94 – 1.84 (2 *m*, 2 H, C^3-H); 1.45 (*s*, 9 H, C$(CH_3)_3$.

^{13}C-NMR (*100 MHz, MeOD*): 175.30 (*s*, 1 C, C^1-^{18}O^{18}OH); 173.85 (*s*, 1 C, C^{5}OO*t*Bu); 158.66 (*s*, 1 C, OCONH); 145.34; 145.18; 142.59 (3 *s*, 4 arom. C); 128.78; 128.17; 126.27; 120.90 (4 *d*, 8 arom. CH); 81.84 (*s*, 1 C, OCMe$_3$); 67.99 (*t*, 1 C, OCH$_2$); 54.54 (*d*, 1 C, NHCH); 48.42 (*s*, 1 C, OCH$_2$*C*H); 32.71 (*t*, 1 C, C^{3}H$_2$); 28.34 (*q*, 3 C, OC(*C*H$_3$)$_3$); 27.99 (*t*, 1 C, C^{4}H$_2$).

MS (- ESI-MS, MeOH + 0.52% NH_3): 857.3 (25, [2 M_{2x} - H]$^-$); 428.1 (100, [M_{2x} – H]$^-$); 426.1 (10, [M_{1x} – H]-); 232.0 (70, [M_{2x} – H – $C_{14}H_{12}O$]$^-$); 230.0 (10, [M_{1x} – H – $C_{14}H_{12}O$]$^-$); 206.0 (79, [M_{2x} – H – $C_{15}H_{10}O_2$]$^-$); 204.1 (11, [M_{1x} – H – $C_{15}H_{10}O_2$]$^-$); 131.9 (41, [M_{2x} – H – $C_{14}H_{11}O$ – $C_5H_9O_2$]$^-$); 130.0 (6, [M_{1x} – H – $C_{14}H_{11}O$ – $C_5H_9O_2$]$^-$).

11.3 Synthesis of Fmoc-L-Trp(Boc)-^{18}OH (**154**)

858 mg dry 3,5-dimethylpyridine hydrobromide (4.56 mmol, 20 equiv.) was suspended in 5 ml dry DMF under N_2, and 437 mg dried EDC.HCl (2.28 mmol, 10 equiv.) and 180 µl $^{18}OH_2$ (9.0 mmol, 40 equiv.) were added. 120 mg dried Fmoc-L-Trp(Boc)-OH (228 µmol), dissolved in 4 ml dry DMF was immediately added by syringe. The mixture was stirred under N_2 at rt for 18 h. A second portion dried EDC.HCl (437 mg, 2.28 mmol, 10 equiv.) was added. The mixture was stirred another 8 h and the third portion dried EDC.HCl (437 mg, 2.28 mmol, 10 equiv.) was added. The solution was stirred for a final 15 hours. The solvent was removed by rv and diluted with AcOEt (30 ml) and washed three times with 0.1 M citric acid and once with 0.1 M citric acid in brine. All aqu. layers were extracted once with AcOEt

(30 ml). The comb. org. layers were dried over $MgSO_4$, filtered, and the solvent removed by rv and hv. The isolated material was partially dissolved in hot toluene (10 ml), filtered and the filtrate dried using rv and hv to furnish 106 mg (200 mmol, 88%) of a colourless solid.

Analytical data for Fmoc-L-Trp(Boc)-^{16}OH (starting material):

$[\alpha]_D^{20}$ (*DMF*): - 19.8
IR (neat): 1726.0*s*; 1518.7*w*; 1450.2*m*; 1375.0*m*; 1333.5*m*; 1260.3*m*; 1159.0*m*; 1085.7*m*; 746.3*m*.

^{1}H-NMR (400 MHz, MeOD): 8.08 (*d*, ^3J = 8.0, 1 H, arom. indole-H); 7.71 (*d*, ^3J = 7.6, 2 H, Fluorenyl-C$^{4'/5'}$-H); 7.60 (*d*, ^3J = 7.6, 1 H, arom. indole-H); 7.51 – 7.48 (*m*, 3 H, arom. indole-H & Fluorenyl-C$^{1'/8'}$-H); 7.33 – 7.16 (*m*, 6 H, Fluorenyl-C$^{2'/3'/6'/7'}$-H & arom. indole-H); 4.58 (*dd*, ^3J = 9.3, ^3J = 4.5, 1 H, C^{2}H); 4.29 – 4.25 (*m*, 1 H, *cis*- & *trans*-form of C$^{9'}$H); 4.17 – 4.08 (*m*, 2 H, *cis*- & *trans*-form of C$^{10'}$H$_2$); 3.32 (*dd*, ^2J = 14.8, ^3J = 4.5, 1 H, C^{3}H$_2$); 3.11 (*dd*, ^2J = 14.8, ^3J = 9.3, 1 H, C^{3}H$_2$); 1.55 (*s*, 9 H, C(CH$_3$)$_3$).

^{13}C-NMR (*100 MHz, MeOD*): 175.30 (*s*, 1 C, C^{1}OOH); 158.37 (*s*, 1 C, OCONH); 150.92 (*s*, 1 C, OCON); 145.14; 145.09; 142.46; 142.43 (4 *s*, 4 Fluorenyl-C); 136.81; 131.76 (2 *s*, 2 arom. indole-C); 128.67; 128.08; 126.27; 126.15; 125.40; 125.04; 123.67; 120.84; 120.81; 119.97 (10 *d*, 10 arom. CH); 117.69 (*s*, 1 arom. indole-C); 116.14 (*d*, 1 arom. indole-CH); 84.67 (*s*, 1 C, OCMe$_3$); 68.05 (*t*, 1 C, C$^{10'}$H$_2$); 55.31 (*d*, 1 C, NHC^{2}H); 48.25 (*d*, 1 C, C$^{9'}$H); 28.71 (*t*, 1 C, C^{3}H$_2$); 28.32 (*q*, 3 C, OC(*C*H$_3$)$_3$).

Analytical data for Fmoc-L-Trp(Boc)-^{18}OH (product):

$[\alpha]_D^{20}$ (*DMF*): - 18.8
IR (neat): 1724.1*s*; 1516.7*w*; 1450.2*m*; 1374.0*m*; 1332.6*m*; 1259.3*m*; 1159.0*m*; 1085.7*m*; 1052.0*m*; 746.3m.

^{1}H-NMR (400 MHz, MeOD): 8.08 (*d*, ^3J = 7.8, 1 H, arom. indole-H); 7.73 (*d*, ^3J = 7.4, 2 H, Fluorenyl-C$^{4'/5'}$-H); 7.60 (*d*, ^3J = 7.5, 1 H, arom. indole-H); 7.55 – 7.50 (*m*, 3 H, arom. indole-H & Fluorenyl-C$^{1'/8'}$-H); 7.34 – 7.12 (*m*, 6 H, Fluorenyl-C$^{2'/3'/6'/7'}$-H & arom. indole-H); 4.58 (*dd*, ^3J = 9.3, ^3J = 4.6, 1 H, C^{2}H); 4.31 – 4.27 (*m*, 1 H, *cis*- & *trans*-form of C$^{9'}$H); 4.19 – 4.12 (*m*, 2 H, *cis*- & *trans*-form of C$^{10'}$H$_2$); 3.31 (*dd*, ^2J = 14.8, ^3J = 4.6, 1 H, C^{3}H$_2$); 3.11 (*dd*, ^2J = 14.8, ^3J = 9.3, 1 H, C^{3}H$_2$); 1.56 (*s*, 9 H, C(CH$_3$)$_3$).

^{13}C-NMR (*100 MHz, MeOD*): 175.10 (*s*, 1 C, C^1-^{18}O^{18}OH); 158.40 (*s*, 1 C, OCONH); 150.94 (*s*, 1 C, OCON); 145.17; 145.13; 142.49; 142.46 (4 *s*, 4 Fluorenyl-C); 136.84; 131.77 (2 *s*, 2 arom. indole-C); 128.70; 128.68; 128.10; 126.24; 126.18; 126.08; 125.42; 125.05; 123.68; 120.85; 120.82; 119.97 (12 *d*, 10 arom. CH); 117.70 (*s*, 1 arom. indole-C); 116.15 (*d*, 1 arom. indole-CH); 84.69 (*s*, 1 C, OCMe$_3$); 68.07 (*t*, 1 C, C$^{10'}$H$_2$); 55.26 (*d*, 1 C, NHC^{2}H); 48.29 (*d*, 1 C, C$^{9'}$H); 28.77 (*t*, 1 C, C^{3}H$_2$); 28.33 (*q*, 3 C, OC(*C*H$_3$)$_3$).

MS (- ESI-MS, MeOH): 529.1 (100, [M_{2x} – H]$^-$); 527.1 (18, [M_{1x} – H]$^-$); 426.9 (9); 391.0 (9); 333.1 (68, [M_{2x} – H – $C_{14}H_{11}O$]$^-$); 331.1 (12, [M_{1x} – H – $C_{14}H_{11}O$]$^-$); 307.1 (72, [M_{2x}

– H – $C_{15}H_{10}O_2$]$^-$); 305.1 (14, [M_{1x} – H – $C_{15}H_{10}O_2$]$^-$); 207.0 (21, [M_{2x} – H – $C_{15}H_{10}O_2$ – $C_5H_8O_2$]$^-$); 190.9 (10); 110.9 (5).

11.4 Synthesis of Fmoc-L-Cys(Trt)-^{18}OH (**155**)

960 mg dry 3,5-dimethylpyridine hydrobromide (5.10 mmol, 20 equiv.) was suspended in 3 ml dry DMF under N_2, and 490 mg of dry EDC.HCl (2.56 mmol, 10 equiv.) and 256 µl $^{18}OH_2$ (12.8 mmol, 50 equiv.) were added. 150 mg of dry Fmoc-L-Cys(Trt)-OH (256 µmol) dissolved in 3 ml dry DMF was immediately added. The mixture was stirred under N_2 at rt for 18 h. A second portion dry EDC (490 mg, 2.56 mmol, 10 equiv.) was added. The mixture was stirred another 8 h and a third portion dry EDC.HCl (490 mg, 2.56 mmol, 10 equiv.) was added. The solution was stirred for 15 hours, diluted into AcOEt (30 ml), washed twice with 0.1 M citric acid (25 ml) and once with 0.1 M citric acid in brine (25 ml). All aq. layers were back extracted with AcOEt (30 ml) and the comb. org. layers were dried over $MgSO_4$, filtered, and the solvent removed by rv and hv. The isolated material was filtered over SiO_2 using CH_2Cl_2/AcOEt 1:1 as the solvent and dried. The resulting solid was dissolved into approximately 1 ml of AcOEt and hexane was added (15 ml). The milky suspension was cooled to -20°, whereas an oily precipitate for med. The oily precipitate was isolated by decantation and dried under hv to yield 143 mg (243 mmol, 95%) of a colourless solid.

Analytical data for Fmoc-L-Cys(Trt)-^{16}OH (starting material):

$[\alpha]_D^{20}$ (*$CHCl_3$*): + 12.86
IR (neat): 1720.2*s*; 1508.1*m*; 1442.5*m*; 1333.5*w*; 1221.7*m*; 1053.9*m*; 747.3*s*; 702.9*m*; 622.9*w*.

^{1}H-NMR (400 MHz, $CDCl_3$ + MeOD): 7.75 (*dd*, ^3J = 7.3, ^4J = 3.5, 2 H, Fluorenyl-C$^{4'/5'}$-H); 7.61 (*dd*, ^3J = 7.1, ^4J = 3.3, 2 H, Fluorenyl-C$^{1'/8'}$-H); 7.41 – 7.17 (*m*, 19 H, Fluorenyl-C$^{2'/3'/6'/7'}$-H & arom. CH Trt); 4.36 (*d*, ^3J = 7.0, 2 H, C$^{10'}$-H_2); 4.28 – 4.09 (*m*, 2 H, C^2-H & C$^{9'}$-H); 2.68 (*d*, ^3J = 5.6, 2 H, C$^3H_2$).

^{13}C-NMR (*100 MHz, $CDCl_3$ + MeOD*): 172.52 (*s*, 1 C, C^{1}OOH); 156.03 (*s*, 1 C, OCONH); 144.39 (*s*, 3 arom. Trt-C); 143.88; 143.74; 141.29; 141.27 (4 *s*, 4 arom. Fluorenyl-C); 129.54; 127.99; 127.74; 127.73; 127.12; 126.87; 125.14; 125.13; 119.95 (9 *d*, 8 arom. Fluorenyl-CH & 15 arom. Trt-CH); 67.10 (*t*, 1 C, C$^{10'}H_2$); 66.96 (*s*, 1 C, $SCPh_3$); 52.84 (*d*, 1 C, NHCH); 47.09 (*d*, 1 C, C$^{9'}$H); 33.91 (*t*, 1 C, SCH_2).

Analytical data for Fmoc-L-Cys(Trt)-^{18}OH (product):

$[\alpha]_D^{20}$ (*$CHCl_3$*): + 13.06
IR (neat): 1700.9*s*; 1507.1*m*; 1443.5*m*; 1331.6*w*; 1221.7*m*; 1052.9*m*; 748.2*s*; 702.9*m*; 622.9*w*.

^{1}H-NMR (400 MHz, $CDCl_3$ + MeOD): 7.77 – 7.75 (*m*, 2 H, Fluorenyl-C$^{4'/5'}$-H); 7.61 (br. *s*, 2 H, Fluorenyl-C$^{1'/8'}$-H); 7.41 – 7.18 (*m*, 19 H, Fluorenyl-C$^{2'/3'/6'/7'}$-H & arom. CH Trt); 4.36 (*d*, ^3J = 6.6, 2 H, C$^{10'}$-H_2); 4.26 – 4.15 (*m*, 2 H, C^2-H & C$^{9'}$-H); 2.68 (*d*, ^3J = 5.2, 2 H, C$^3H_2$).

^{13}C-NMR (*100 MHz, $CDCl_3$ + MeOD*): 172.37 (*s*, 1 C, C^{1}-$^{18}O^{18}OH$); 156.05 (*s*, 1 C, OCONH); 144.38 (*s*, 3 arom. Trt-C); 143.86; 143.72; 141.26 (3 *s*, 4 arom. Fluorenyl-C); 129.53; 127.96; 127.71; 127.10; 126.84; 125.11; 119.93 (7 *d*, 8 arom. Fluorenyl-CH & 15 arom. Trt-CH); 67.07 (*t*, 1 C, $C^{10'}H_2$); 66.92 (*s*, 1 C, $SCPh_3$); 52.81 (*d*, 1 C, NHCH); 47.07 (*d*, 1 C, $C^{9'}H$); 33.91 (*t*, 1 C, SCH_2).

MS (- ESI-MS. MeOH + NaI): 1177.4 (9, [2 M_{2x} - H]$^-$); 588.1 (65, [M_{2x} – H]$^-$); 586.1 (7, [M_{1x} – H]$^-$); 392.1 (33, [M_{2x} – H – $C_{14}H_{11}O$]$^-$); 366.1 (60, [M_{2x} – H – $C_{15}H_{10}O_2$]$^-$); 364.1 (5, [M_{1x} – H – $C_{15}H_{10}O_2$]$^-$); 275.0 (100, [Ph_3CS]$^-$); 243.0 (5, [Ph_3C]$^-$).

Part IV

Appendix

12. Crystallographic data

Table 12.1. *Crystallographic Data of* ***42***

Crystallised from	dioxane / pentane
Empirical formula	$C_{19}H_{25}N_3O_5$
Formula weight [g mol^{-1}]	375.42
Crystal colour, habit	colourless, prism
Crystal dimensions [mm]	$0.12 \times 0.25 \times 0.30$
Temperature [K]	160(1)
Crystal system	monoclinic
Space group	$P2_1/c$ (#14)
Z	4
Reflections for cell determination	5672
2θ range for cell determination [°]	4 – 60
Unit cell parameters a [Å]	9.2486(2)
b [Å]	23.8477(8)
c [Å]	10.0247(3)
α [°]	90
β [°]	116.666(2)
γ [°]	90
V [Å^3]	1975.9(1)
F(000)	800
D_X [g cm^{-3}]	1.262
μ(Mo $K\alpha$) [mm^{-1}]	0.0921
Scan type	ϕ and ω
2θ(max) [°]	60
Total reflections measured	39772
Symmetry independent reflections	5719
R_{int}	0.067
Reflections with $I > 2\sigma(I)$	3851
Reflections used in refinement	5718
Parameters refined	256
Final $R(F)$ [$I > 2\sigma(I)$ reflections]	0.0747
$wR(F^2)$ (all data)	0.2209
Weights:	$w = [\sigma^2(F_o^2) + (0.0939P)^2 + 1.7573P]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$
Goodness of fit	1.044
Final Δ_{max}/σ	0.001
$\Delta\rho$ (max; min) [e Å^{-3}]	1.03; -0.35
$\sigma(d_{(C-C)})$ [Å]	0.003 – 0.005

Table 12.2. *Crystallographic Data of* ***89***

Crystallised from	EtOAc / CH_2Cl_2
Empirical formula	$C_{19}H_{24}N_2O_5S$
Formula weight [g mol^{-1}]	392.47
Crystal colour, habit	colourless, prism
Crystal dimensions [mm]	$0.15 \times 0.25 \times 0.50$
Temperature [K]	160(1)
Crystal system	monoclinic
Space group	$P2_1/c$ (#14)
Z	4
Reflections for cell determination	30126
2θ range for cell determination [°]	4 – 50
Unit cell parameters a [Å]	13.9062(5)
b [Å]	9.7100(5)
c [Å]	15.3213(5)
α [°]	90
β [°]	103.880(3)
γ [°]	90
V [Å^3]	2008.4(1)
F(000)	832
D_X [g cm^{-3}]	1.298
μ(Mo $K\alpha$) [mm^{-1}]	0.193
Scan type	ω
2θ(max) [°]	50
Transmission factors (min; max)	0.663; 0.981
Total reflections measured	26977
Symmetry independent reflections	3542
R_{int}	0.109
Reflections with $I > 2\sigma(I)$	2842
Reflections used in refinement	3542
Parameters refined	254
Final $R(F)$ [$I > 2\sigma(I)$ reflections]	0.0551
$wR(F^2)$ (all data)	0.1244
Weights:	$w = [\sigma^2(F_O^2) + (0.0375P)^2 + 1.7222P]^{-1}$ where $P = (F_O^2 + 2F_C^2)/3$
Goodness of fit	1.125
Secondary extinction coefficient	0.010(2)
Final Δ_{max}/σ	0.001
$\Delta\rho$ (max; min) [e Å^{-3}]	0.28; -0.22
$\sigma(d_{(C-C)})$ [Å]	0.003 – 0.005

13. Literature

Literature

[1] I. Bang, *Biochemische Zeitschrift* **1910**, *26*, 293.
[2] M. Gellert, M. N. Lipsett, D. R. Davies, *Proc Natl Acad Sci U S A* **1962**, *48*, 2013.
[3] A. N. Lane, J. B. Chaires, R. D. Gray, J. O. Trent, *Nucleic Acids Research* **2008**, *36*, 5482.
[4] G. N. Parkinson, Royal Society of Chemistry, **2006**, pp. 1.
[5] D. Sen, W. Gilbert, *Nature (London, United Kingdom)* **1988**, *334*, 364.
[6] W. I. Sundquist, A. Klug, *Nature (London, United Kingdom)* **1989**, *342*, 825.
[7] S. Burge, G. N. Parkinson, P. Hazel, A. K. Todd, S. Neidle, *Nucleic Acids Research* **2006**, *34*, 5402.
[8] S. Neidle, *Current Opinion in Structural Biology* **2009**, *19*, 239.
[9] T. Simonsson, *Biological Chemistry* **2001**, *382*, 621.
[10] N. V. Hud, F. W. Smith, F. A. L. Anet, J. Feigon, *Biochemistry* **1996**, *35*, 15383.
[11] A. K. Todd, M. Johnston, S. Neidle, *Nucleic Acids Research* **2005**, *33*, 2901.
[12] J. L. Huppert, *Chemical Society Reviews* **2008**, *37*, 1375.
[13] J. L. Huppert, S. Balasubramanian, *Nucleic Acids Research* **2005**, *33*, 2908.
[14] C. Schaffitzel, I. Berger, J. Postberg, J. Hanes, H. J. Lipps, A. Plueckthun, *Proceedings of the National Academy of Sciences of the United States of America* **2001**, *98*, 8572.
[15] L. Oganesian, T. M. Bryan, *BioEssays* **2007**, *29*, 155.
[16] J.-L. Mergny, J.-F. Riou, P. Maillieet, M.-P. Teulade-Richou, E. Gilson, *Nucleic Acids Research* **2002**, *30*, 839.
[17] M. T. P. Gilbert, J. Binladen, W. Miller, C. Wiuf, E. Willerslev, H. Poinar, J. E. Carlson, J. H. Leebens-Mack, S. C. Schuster, *Nucleic Acids Research* **2007**, *35*, 1.
[18] K. Guo, A. Pourpak, K. Beetz-Rogers, V. Gokhale, D. Sun, L. H. Hurley, *Journal of the American Chemical Society* **2007**, *129*, 10220.
[19] A. K. Shukla, K. B. Roy, *Biological Chemistry* **2006**, *387*, 251.
[20] F. Boan, J. Gomez-Marquez, *ChemBioChem* **2010**, *11*, 331.
[21] P. Mani, V. K. Yadav, S. K. Das, S. Chowdhury, *PloS one* **2009**, *4*, e4399.
[22] L. A. Hanakahi, H. Sun, N. Maizels, *Journal of Biological Chemistry* **1999**, *274*, 15908.
[23] C. De, Anne, L. Lacroix, C. Douarre, N. Temime-Smaali, C. Trentesaux, J.-F. Riou, J.-L. Mergny, *Biochimie* **2008**, *90*, 131.
[24] S. Neidle, G. N. Parkinson, *Current Opinion in Structural Biology* **2003**, *13*, 275.
[25] A. M. Zahler, J. R. Williamson, T. R. Cech, D. M. Prescott, *Nature (London, United Kingdom)* **1991**, *350*, 718.
[26] S. Neidle, M. A. Read, *Biopolymers* **2001**, *56*, 195.
[27] J. L. Huppert, *FEBS Journal* **2010**, *277*, 3452.
[28] W. E. Wright, V. M. Tesmer, K. E. Huffman, S. D. Levene, J. W. Shay, *Genes & Development* **1997**, *11*, 2801.
[29] Y. Wang, D. J. Patel, *Structure (Cambridge, MA, United States)* **1993**, *1*, 263.
[30] G. N. Parkinson, M. P. H. Lee, S. Neidle, *Nature (London, United Kingdom)* **2002**, *417*, 876.
[31] A. Smogorzewska, L. de, Titia., *Annual Review of Biochemistry* **2004**, *73*, 177.

[32] A. J. Zaug, E. R. Podell, T. R. Cech, *Proceedings of the National Academy of Sciences of the United States of America* **2005**, *102*, 10864.
[33] D. Gomez, M.-F. O'Donohue, T. Wenner, C. Douarre, J. Macadre, P. Koebel, M.-J. Giraud-Panis, H. Kaplan, A. Kolkes, K. Shin-ya, J.-F. Riou, *Cancer Research* **2006**, *66*, 6908.
[34] J. L. Huppert, S. Balasubramanian, *Nucleic Acids Research* **2007**, *35*, 406.
[35] J. Eddy, N. Maizels, *Nucleic Acids Research* **2008**, *36*, 1321.
[36] S. Saxonov, P. Berg, D. L. Brutlag, *Proceedings of the National Academy of Sciences of the United States of America* **2006**, *103*, 1412.
[37] G. Strathdee, A. Sim, R. Brown, *Biochemical Society Transactions* **2004**, *32*, 913.
[38] M. Bina, P. Wyss, W. Ren, W. Szpankowski, E. Thomas, R. Randhawa, S. Reddy, P. M. John, E. I. Pares-Matos, A. Stein, H. Xu, S. A. Lazarus, *Genomics* **2004**, *84*, 929.
[39] X. Xie, J. Lu, E. J. Kulbokas, T. R. Golub, V. Mootha, K. Lindblad-Toh, E. S. Lander, M. Kellis, *Nature (London, United Kingdom)* **2005**, *434*, 338.
[40] V. Matys, O. V. Kel-Margoulis, E. Fricke, I. Liebich, S. Land, A. Barre-Dirrie, I. Reuter, D. Chekmenev, M. Krull, K. Hornischer, N. Voss, P. Stegmaier, B. Lewicki-Potapov, H. Saxel, A. E. Kel, E. Wingender, *Nucleic Acids Research* **2006**, *34*, D108.
[41] O. Sakatsume, H. Tsutsui, Y. Wang, H. Gao, X. Tang, T. Yamauchi, K. Itakura, K. K. Yokoyama, T. Murata, *Journal of Biological Chemistry* **1996**, *271*, 31322.
[42] E. H. Postel, S. E. Mango, S. J. Flint, *Molecular and Cellular Biology* **1989**, *9*, 5123.
[43] U. Siebenlist, L. Hennighausen, J. Battey, P. Leder, *Cell (Cambridge, MA, United States)* **1984**, *37*, 381.
[44] D. M. L. Morgan, C. P. D. Wheeler-Jones, *Biochemical Society Transactions* **1998**, *26*, S367.
[45] T. Tomonaga, D. Levens, *Proceedings of the National Academy of Sciences of the United States of America* **1996**, *93*, 5830.
[46] T. Simonsson, P. Pecinka, M. Kubista, *Nucleic Acids Research* **1998**, *26*, 1167.
[47] C. L. Grand, H. Han, R. M. Munoz, S. Weitman, H. Von, Daniel D., L. H. Hurley, D. J. Bearss, *Molecular Cancer Therapeutics* **2002**, *1*, 565.
[48] H. Han, D. R. Langley, A. Rangan, L. H. Hurley, *Journal of the American Chemical Society* **2001**, *123*, 8902.
[49] Y. Qin, L. H. Hurley, *Biochimie* **2008**, *90*, 1149.
[50] Y. Kohwi, Y. Panchenko, *Genes & Development* **1993**, *7*, 1766.
[51] S. Myers, L. Bottolo, C. Freeman, G. McVean, P. Donnelly, *Science (Washington, DC, United States)* **2005**, *310*, 321.
[52] L. A. Cahoon, H. S. Seifert, *Science (Washington, DC, United States)* **2009**, *325*, 764.
[53] Y. N. Osheim, E. B. Mougey, J. Windle, M. Anderson, O'Reilly, O. L. Miller, Jr., A. Beyer, B. Sollner-Webb, *Journal of Cell Biology* **1996**, *133*, 943.
[54] R. A. Marciniak, D. B. Lombard, F. B. Johnson, L. Guarente, *Proceedings of the National Academy of Sciences of the United States of America* **1998**, *95*, 6887.
[55] M. D. Gray, L. Wang, H. Youssoufian, G. M. Martin, J. Oshima, *Experimental Cell Research* **1998**, *242*, 487.

[56] J. Alzeer, B. R. Vummidi, P. J. C. Roth, N. W. Luedtke, *Angewandte Chemie, International Edition* **2009**, *48*, 9362.
[57] I. M. Dixon, F. Lopez, A. M. Tejera, J.-P. Esteve, M. A. Blasco, G. Pratviel, B. Meunier, *Journal of the American Chemical Society* **2007**, *129*, 1502.
[58] F. Rosu, V. Gabelica, K. Shin-ya, P. De, Edwin., *Chemical Communications (Cambridge, United Kingdom)* **2003**, 2702.
[59] J. Kypr, I. Kejnovska, D. Renciuk, M. Vorlickova, *Nucleic Acids Research* **2009**, *37*, 1713.
[60] R. T. Wheelhouse, S. A. Jennings, V. A. Phillips, D. Pletsas, P. M. Murphy, N. C. Garbett, J. B. Chaires, T. C. Jenkins, *Journal of Medicinal Chemistry* **2006**, *49*, 5187.
[61] F. Rosu, P. De, Edwin, L. Guittat, P. Alberti, L. Lacroix, P. Mailliet, J.-F. Riou, J.-L. Mergny, *Biochemistry* **2003**, *42*, 10361.
[62] J. Ren, J. B. Chaires, *Biochemistry* **1999**, *38*, 16067.
[63] N. W. Kim, M. A. Piatyszek, K. R. Prowse, C. B. Harley, M. D. West, P. L. C. Ho, G. M. Coviello, W. E. Wright, S. L. Weinrich, J. W. Shay, *Science (Washington, D. C.)* **1994**, *266*, 2011.
[64] J. Reed, M. Gunaratnam, M. Beltran, A. P. Reszka, R. Vilar, S. Neidle, *Analytical Biochemistry* **2008**, *380*, 99.
[65] T.-m. Ou, Y.-j. Lu, J.-h. Tan, Z.-s. Huang, K.-Y. Wong, L.-q. Gu, *ChemMedChem* **2008**, *3*, 690.
[66] D. Monchaud, M.-P. Teulade-Fichou, *Organic & Biomolecular Chemistry* **2008**, *6*, 627.
[67] M. C. Nielsen, T. Ulven, *Current Medicinal Chemistry* **2010**, *17*, 3438.
[68] D. Sun, B. Thompson, B. E. Cathers, M. Salazar, S. M. Kerwin, J. O. Trent, T. C. Jenkins, S. Neidle, L. H. Hurley, *Journal of Medicinal Chemistry* **1997**, *40*, 2113.
[69] P. J. Perry, A. P. Reszka, A. A. Wood, M. A. Read, S. M. Gowan, H. S. Dosanjh, J. O. Trent, T. C. Jenkins, L. R. Kelland, S. Neidle, *Journal of Medicinal Chemistry* **1998**, *41*, 4873.
[70] P. J. Perry, S. M. Gowan, A. P. Reszka, P. Polucci, T. C. Jenkins, L. R. Kelland, S. Neidle, *Journal of Medicinal Chemistry* **1998**, *41*, 3253.
[71] P. J. Perry, M. A. Read, R. T. Davies, S. M. Gowan, A. P. Reszka, A. A. Wood, L. R. Kelland, S. Neidle, *Journal of Medicinal Chemistry* **1999**, *42*, 2679.
[72] M. A. Read, A. A. Wood, J. R. Harrison, S. M. Gowan, L. R. Kelland, H. S. Dosanjh, S. Neidle, *Journal of Medicinal Chemistry* **1999**, *42*, 4538.
[73] R. J. Harrison, S. M. Gowan, L. R. Kelland, S. Neidle, *Bioorganic & Medicinal Chemistry Letters* **1999**, *9*, 2463.
[74] R. J. Harrison, J. Cuesta, G. Chessari, M. A. Read, S. K. Basra, A. P. Reszka, J. Morrell, S. M. Gowan, C. M. Incles, F. A. Tanious, W. D. Wilson, L. R. Kelland, S. Neidle, *Journal of Medicinal Chemistry* **2003**, *46*, 4463.
[75] S. M. Haider, G. N. Parkinson, S. Neidle, *Journal of Molecular Biology* **2003**, *326*, 117.
[76] M. J. B. Moore, C. M. Schultes, J. Cuesta, F. Cuenca, M. Gunaratnam, F. A. Tanious, W. D. Wilson, S. Neidle, *Journal of Medicinal Chemistry* **2006**, *49*, 582.
[77] J. Stanslas, D. J. Hagan, M. J. Ellis, C. Turner, J. Carmichael, W. Ward, T. R. Hammonds, M. F. G. Stevens, *Journal of Medicinal Chemistry* **2000**, *43*, 1563.
[78] S. M. Gowan, R. Heald, M. F. G. Stevens, L. R. Kelland, *Molecular Pharmacology* **2001**, *60*, 981.

[79] R. A. Heald, C. Modi, J. C. Cookson, I. Hutchinson, C. A. Laughton, S. M. Gowan, L. R. Kelland, M. F. G. Stevens, *Journal of Medicinal Chemistry* **2002**, *45*, 590.
[80] J. C. Cookson, R. A. Heald, M. F. G. Stevens, *Journal of Medicinal Chemistry* **2005**, *48*, 7198.
[81] J. C. Cookson, F. Dai, V. Smith, R. A. Heald, C. A. Laughton, M. F. G. Stevens, A. M. Burger, *Molecular Pharmacology* **2005**, *68*, 1551.
[82] E. Gavathiotis, R. A. Heald, M. F. G. Stevens, M. S. Searle, *Angewandte Chemie, International Edition* **2001**, *40*, 4749.
[83] E. Gavathiotis, R. A. Heald, M. F. G. Stevens, M. S. Searle, *Journal of Molecular Biology* **2003**, *334*, 25.
[84] P. J. Perry, S. M. Gowan, M. A. Read, L. R. Kelland, S. Neidle, *Anti-Cancer Drug Design* **1999**, *14*, 373.
[85] V. Caprio, B. Guyen, Y. Opoku-Boahen, J. Mann, S. Gowan, L. M. Kelland, M. A. Read, S. Neidle, *Bioorganic & Medicinal Chemistry Letters* **2000**, *10*, 2063.
[86] B. Guyen, C. M. Schultes, P. Hazel, J. Mann, S. Neidle, *Organic & Biomolecular Chemistry* **2004**, *2*, 981.
[87] J.-M. Zhou, X.-F. Zhu, Y.-J. Lu, R. Deng, Z.-S. Huang, Y.-P. Mei, Y. Wang, W.-L. Huang, Z.-C. Liu, L.-Q. Gu, Y.-X. Zeng, *Oncogene* **2006**, *25*, 503.
[88] J.-L. Zhou, Y.-J. Lu, T.-M. Ou, J.-M. Zhou, Z.-S. Huang, X.-F. Zhu, C.-J. Du, X.-Z. Bu, L. Ma, L.-Q. Gu, Y.-M. Li, A. S.-C. Chan, *Journal of Medicinal Chemistry* **2005**, *48*, 7315.
[89] T.-M. Ou, Y.-J. Lu, C. Zhang, Z.-S. Huang, X.-D. Wang, J.-H. Tan, Y. Chen, D.-L. Ma, K.-Y. Wong, J. C.-O. Tang, A. S.-C. Chan, L.-Q. Gu, *Journal of Medicinal Chemistry* **2007**, *50*, 1465.
[90] J.-L. Mergny, L. Lacroix, M.-P. Teulade-Fichou, C. Hounsou, L. Guittat, M. Hoarau, P. B. Arimondo, J.-P. Vigneron, J.-M. Lehn, J.-F. Riou, T. Garestier, C. Helene, *Proceedings of the National Academy of Sciences of the United States of America* **2001**, *98*, 3062.
[91] M.-P. Teulade-Fichou, C. Carrasco, L. Guittat, C. Bailly, P. Alberti, J.-L. Mergny, A. David, J.-M. Lehn, W. D. Wilson, *Journal of the American Chemical Society* **2003**, *125*, 4732.
[92] H. Bertrand, S. Bombard, D. Monchaud, M.-P. Teulade-Fichou, *JBIC, Journal of Biological Inorganic Chemistry* **2007**, *12*, 1003.
[93] O. Y. Fedoroff, M. Salazar, H. Han, V. V. Chemeris, S. M. Kerwin, L. H. Hurley, *Biochemistry* **1998**, *37*, 12367.
[94] H. Han, C. L. Cliff, L. H. Hurley, *Biochemistry* **1999**, *38*, 6981.
[95] A. Rangan, O. Y. Fedoroff, L. H. Hurley, *Journal of Biological Chemistry* **2001**, *276*, 4640.
[96] I. Naasani, H. Seimiya, T. Yamori, T. Tsuruo, *Cancer Research* **1999**, *59*, 4004.
[97] M. Franceschin, L. Rossetti, A. D'Ambrosio, S. Schirripa, A. Bianco, G. Ortaggi, M. Savino, C. Schultes, S. Neidle, *Bioorganic & Medicinal Chemistry Letters* **2006**, *16*, 1707.
[98] W.-J. Zhang, T.-M. Ou, Y.-J. Lu, Y.-Y. Huang, W.-B. Wu, Z.-S. Huang, J.-L. Zhou, K.-Y. Wong, L.-Q. Gu, *Bioorganic & Medicinal Chemistry* **2007**, *15*, 5493.
[99] J.-Y. Fan, D. Sun, H. Yu, S. M. Kerwin, L. H. Hurley, *Journal of Medicinal Chemistry* **1995**, *38*, 408.

[100] W. Duan, A. Rangan, H. Vankayalapati, M.-Y. Kim, Q. Zeng, D. Sun, H. Han, O. Y. Fedoroff, D. Nishioka, S. Y. Rha, E. Izbicka, H. Von, Daniel D., L. H. Hurley, *Molecular Cancer Therapeutics* **2001**, *1*, 103.
[101] A. K. Mehta, Y. Shayo, H. Vankayalapati, L. H. Hurley, J. Schaefer, *Biochemistry* **2004**, *43*, 11953.
[102] M.-Y. Kim, W. Duan, M. Gleason-Guzman, L. H. Hurley, *Journal of Medicinal Chemistry* **2003**, *46*, 571.
[103] J. C. Wang, *Annual Review of Biochemistry* **1996**, *65*, 635.
[104] R. J. Reece, A. Maxwell, *Critical Reviews in Biochemistry and Molecular Biology* **1991**, *26*, 335.
[105] D. Drygin, A. Siddiqui-Jain, S. O'Brien, M. Schwaebe, A. Lin, J. Bliesath, C. B. Ho, C. Proffitt, K. Trent, J. P. Whitten, J. K. C. Lim, H. Von, Daniel, K. Anderes, W. G. Rice, *Cancer Research* **2009**, *69*, 7653.
[106] S. Balasubramanian, L. H. Hurley, S. Neidle, *Nature Reviews Drug Discovery* **2011**, *10*, 261.
[107] A. D. Moorhouse, A. M. Santos, M. Gunaratnam, M. Moore, S. Neidle, J. E. Moses, *Journal of the American Chemical Society* **2006**, *128*, 15972.
[108] G. Pennarun, C. Granotier, L. R. Gauthier, D. Gomez, F. Hoffschir, E. Mandine, J.-F. Riou, J.-L. Mergny, P. Mailliet, F. D. Boussin, *Oncogene* **2005**, *24*, 2917.
[109] C. De, Anne, E. DeLemos, J.-L. Mergny, M.-P. Teulade-Fichou, D. Monchaud, *Journal of the American Chemical Society* **2007**, *129*, 1856.
[110] J. F. Riou, L. Guittat, P. Mailliet, A. Laoui, E. Renou, O. Petitgenet, F. Megnin-Chanet, C. Helene, J. L. Mergny, *Proceedings of the National Academy of Sciences of the United States of America* **2002**, *99*, 2672.
[111] H. Sun, Y. Tang, J. Xiang, G. Xu, Y. Zhang, H. Zhang, L. Xu, *Bioorganic & Medicinal Chemistry Letters* **2006**, *16*, 3586.
[112] H. Sun, J. Xiang, Y. Tang, G. Xu, *Biochemical and Biophysical Research Communications* **2007**, *352*, 942.
[113] Q. Xu, H. Guo, A. Wlodawer, H. Guo, *Journal of the American Chemical Society* **2006**, *128*, 5994.
[114] H. Bertrand, D. Monchaud, C. De, Anne, R. Guillot, J.-L. Mergny, M.-P. Teulade-Fichou, *Organic & Biomolecular Chemistry* **2007**, *5*, 2555.
[115] R. T. Wheelhouse, D. Sun, H. Han, F. X. Han, L. H. Hurley, *Journal of the American Chemical Society* **1998**, *120*, 3261.
[116] C. Wei, G. Jia, J. Zhou, G. Han, C. Li, *Physical Chemistry Chemical Physics* **2009**, *11*, 4025.
[117] L.-P. Bai, M. Hagihara, Z.-H. Jiang, K. Nakatani, *ChemBioChem* **2008**, *9*, 2583.
[118] L. Martino, B. Pagano, I. Fotticchia, S. Neidle, C. Giancola, *Journal of Physical Chemistry B* **2009**, *113*, 14779.
[119] H. Mita, T. Ohyama, Y. Tanaka, Y. Yamamoto, *Biochemistry* **2006**, *45*, 6765.
[120] H. Zhang, X. Xiao, P. Wang, S. Pang, F. Qu, X. Ai, J. Zhang, *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy* **2009**, *74A*, 243.
[121] H.-J. Zhang, X.-F. Wang, P. Wang, X.-C. Ai, J.-P. Zhang, *Photochemical & Photobiological Sciences* **2008**, *7*, 948.
[122] G. N. Parkinson, R. Ghosh, S. Neidle, *Biochemistry* **2007**, *46*, 2390.
[123] P. Wang, L. Ren, H. He, F. Liang, X. Zhou, Z. Tan, *ChemBioChem* **2006**, *7*, 1155.
[124] F. X. Han, R. T. Wheelhouse, L. H. Hurley, *Journal of the American Chemical Society* **1999**, *121*, 3561.

[125] S. Y. Rha, E. Izbicka, R. Lawrence, K. Davidson, D. Sun, M. P. Moyer, G. D. Roodman, L. Hurley, H. Von, Daniel., *Clinical Cancer Research* **2000**, *6*, 987.
[126] L. H. Hurley, H. Von, Daniel D., A. Siddiqui-Jain, D. Yang, *Seminars in Oncology* **2006**, *33*, 498.
[127] A. Siddiqui-Jain, C. L. Grand, D. J. Bearss, L. H. Hurley, *Proceedings of the National Academy of Sciences of the United States of America* **2002**, *99*, 11593.
[128] Y. Mikami-Terao, M. Akiyama, Y. Yuza, T. Yanagisawa, O. Yamada, H. Yamada, *Cancer Letters (Amsterdam, Netherlands)* **2008**, *261*, 226.
[129] T. del, Miquel, P. Bucek, A. Avino, J. Jaumot, C. Gonzalez, R. Eritja, R. Gargallo, *Biochimie* **2009**, *91*, 894.
[130] Y. Mikami-Terao, M. Akiyama, Y. Yuza, T. Yanagisawa, O. Yamada, T. Kawano, M. Agawa, H. Ida, H. Yamada, *Experimental Eye Research* **2009**, *89*, 200.
[131] C. Wei, L. Wang, G. Jia, J. Zhou, G. Han, C. Li, *Biophysical Chemistry* **2009**, *143*, 79.
[132] C. Romera, O. Bombarde, R. Bonnet, D. Gomez, P. Dumy, P. Calsou, J.-F. Gwan, J.-H. Lin, E. Defrancq, G. Pratviel, *Biochimie* **2011**, *93*, 1310.
[133] D. P. N. Goncalves, S. Ladame, S. Balasubramanian, J. K. M. Sanders, *Organic & Biomolecular Chemistry* **2006**, *4*, 3337.
[134] E. S. Baker, J. T. Lee, J. L. Sessler, M. T. Bowers, *Journal of the American Chemical Society* **2006**, *128*, 2641.
[135] H. Arthanari, S. Basu, T. L. Kawano, P. H. Bolton, *Nucleic Acids Research* **1998**, *26*, 3724.
[136] X. Wu, N. Maizels, *Nucleic Acids Research* **2001**, *29*, 1765.
[137] N. V. Anantha, M. Azam, R. D. Sheardy, *Biochemistry* **1998**, *37*, 2709.
[138] J. Seenisamy, S. Bashyam, V. Gokhale, H. Vankayalapati, D. Sun, A. Siddiqui-Jain, N. Streiner, K. Shinya, E. White, W. D. Wilson, L. H. Hurley, *Journal of the American Chemical Society* **2005**, *127*, 2944.
[139] E. M. Rezler, J. Seenisamy, S. Bashyam, M.-Y. Kim, E. White, W. D. Wilson, L. H. Hurley, *Journal of the American Chemical Society* **2005**, *127*, 9439.
[140] L. Ren, A. Zhang, J. Huang, P. Wang, X. Weng, L. Zhang, F. Liang, Z. Tan, X. Zhou, *ChemBioChem* **2007**, *8*, 775.
[141] L. Zhang, J. Huang, L. Ren, M. Bai, L. Wu, B. Zhai, X. Zhou, *Bioorganic & Medicinal Chemistry* **2008**, *16*, 303.
[142] A. Membrino, M. Paramasivam, S. Cogoi, J. Alzeer, N. W. Luedtke, L. E. Xodo, *Chemical Communications (Cambridge, United Kingdom)* **2010**, *46*, 625.
[143] J. Alzeer, N. W. Luedtke, *Biochemistry* **2010**, *49*, 4339.
[144] K. Shin-ya, K. Wierzba, K.-i. Matsuo, T. Ohtani, Y. Yamada, K. Furihata, Y. Hayakawa, H. Seto, *Journal of the American Chemical Society* **2001**, *123*, 1262.
[145] C. De, Anne, G. Cristofari, P. Reichenbach, L. De, Elsa, D. Monchaud, M.-P. Teulade-Fichou, K. Shin-ya, L. Lacroix, J. Lingner, J.-L. Mergny, *Proceedings of the National Academy of Sciences of the United States of America* **2007**, *104*, 17347.
[146] M.-Y. Kim, M. Gleason-Guzman, E. Izbicka, D. Nishioka, L. H. Hurley, *Cancer Research* **2003**, *63*, 3247.
[147] H. Tahara, K. Shin-Ya, H. Seimiya, H. Yamada, T. Tsuruo, T. Ide, *Oncogene* **2006**, *25*, 1955.

[148] N. Temime-Smaali, L. Guittat, A. Sidibe, K. Shin-ya, C. Trentesaux, J.-F. Riou, *PloS one* **2009**, *4*, e6919.
[149] N. Arnoult, K. Shin-Ya, J. A. Londono-Vallejo, *Cytogenetic and Genome Research* **2008**, *122*, 229.
[150] N. Endoh, K. Tsuboi, R. Kim, Y. Yonezawa, C.-g. Shin, *Heterocycles* **2003**, *60*, 1567.
[151] C. M. Marson, M. Saadi, *Organic & Biomolecular Chemistry* **2006**, *4*, 3892.
[152] S. K. Chattopadhyay, S. Biswas, B. K. Pal, *Synthesis* **2006**, 1289.
[153] S. K. Chattopadhyay, S. Biswas, *Tetrahedron Letters* **2006**, *47*, 7897.
[154] H. Araki, T. Katoh, M. Inoue, *Tetrahedron Letters* **2007**, *48*, 3713.
[155] J. Deeley, A. Bertram, G. Pattenden, *Organic & Biomolecular Chemistry* **2008**, *6*, 1994.
[156] S. K. Chattopadhyay, S. Biswas, S. K. Ghosh, *Synthesis* **2008**, 1029.
[157] T. Doi, M. Yoshida, K. Shin-ya, T. Takahashi, *Organic Letters* **2006**, *8*, 4165.
[158] G. S. Minhas, D. S. Pilch, J. E. Kerrigan, E. J. LaVoie, J. E. Rice, *Bioorganic & Medicinal Chemistry Letters* **2006**, *16*, 3891.
[159] C. M. Barbieri, A. R. Srinivasan, S. G. Rzuczek, J. E. Rice, E. J. LaVoie, D. S. Pilch, *Nucleic Acids Research* **2007**, *35*, 3272.
[160] D. S. Pilch, C. M. Barbieri, S. G. Rzuczek, E. J. LaVoie, J. E. Rice, *Biochimie* **2008**, *90*, 1233.
[161] M. Satyanarayana, S. G. Rzuczek, E. J. LaVoie, D. S. Pilch, A. Liu, L. F. Liu, J. E. Rice, *Bioorganic & Medicinal Chemistry Letters* **2008**, *18*, 3802.
[162] M. Tera, Y. Sohtome, H. Ishizuka, T. Doi, M. Takagi, K. Shin-ya, K. Nagasawa, *Heterocycles* **2006**, *69*, 505.
[163] S. G. Rzuczek, D. S. Pilch, E. J. LaVoie, J. E. Rice, *Bioorganic & Medicinal Chemistry Letters* **2008**, *18*, 913.
[164] M. Tera, H. Ishizuka, M. Takagi, M. Suganuma, K. Shin-ya, K. Nagasawa, *Angewandte Chemie, International Edition* **2008**, *47*, 5557.
[165] M. Satyanarayana, Y.-A. Kim, S. G. Rzuczek, D. S. Pilch, A. A. Liu, L. F. Liu, J. E. Rice, V. La, Edmond J., *Bioorganic & Medicinal Chemistry Letters* **2010**, *20*, 3150.
[166] M. Tera, K. Iida, H. Ishizuka, M. Takagi, M. Suganuma, T. Doi, K. Shin-ya, K. Nagasawa, *ChemBioChem* **2009**, *10*, 431.
[167] K. Iida, M. Tera, T. Hirokawa, K. Shin-ya, K. Nagasawa, *Chemical Communications (Cambridge, United Kingdom)* **2009**, 6481.
[168] K. Jantos, R. Rodriguez, S. Ladame, P. S. Shirude, S. Balasubramanian, *Journal of the American Chemical Society* **2006**, *128*, 13662.
[169] A. Bugaut, K. Jantos, J.-L. Wietor, R. Rodriguez, J. K. M. Sanders, S. Balasubramanian, *Angewandte Chemie, International Edition* **2008**, *47*, 2677.
[170] T. K. Chakraborty, A. Arora, S. Roy, N. Kumar, S. Maiti, *Journal of Medicinal Chemistry* **2007**, *50*, 5539.
[171] P. S. Shirude, E. R. Gillies, S. Ladame, F. Godde, K. Shin-ya, I. Huc, S. Balasubramanian, *Journal of the American Chemical Society* **2007**, *129*, 11890.
[172] P. V. Jena, P. S. Shirude, B. Okumus, K. Laxmi-Reddy, F. Godde, I. Huc, S. Balasubramanian, T. Ha, *Journal of the American Chemical Society* **2009**, *131*, 12522.
[173] S. G. Rzuczek, D. S. Pilch, A. Liu, L. Liu, V. La, Edmond J., J. E. Rice, *Journal of Medicinal Chemistry* **2010**, *53*, 3632.
[174] R. A. Hughes, C. J. Moody, *Angewandte Chemie, International Edition* **2007**, *46*, 7930.

[175] T. Shioiri, K. Ninomiya, S. Yamada, *Journal of the American Chemical Society* **1972**, *94*, 6203.
[176] S.-L. You, H. Razavi, J. W. Kelly, *Angewandte Chemie, International Edition* **2003**, *42*, 83.
[177] M.-Y. Kim, H. Vankayalapati, K. Shin-ya, K. Wierzba, L. H. Hurley, *Journal of the American Chemical Society* **2002**, *124*, 2098.
[178] S. Agrawal, R. P. Ojha, S. Maiti, *Journal of Physical Chemistry B* **2008**, *112*, 6828.
[179] T. T. Charvat, D. J. Lee, W. E. Robinson, A. R. Chamberlin, *Bioorganic & Medicinal Chemistry* **2006**, *14*, 4552.
[180] A. J. Phillips, Y. Uto, P. Wipf, M. J. Reno, D. R. Williams, *Organic Letters* **2000**, *2*, 1165.
[181] V. F. Pozdnev, *Tetrahedron Letters* **1995**, *36*, 7115.
[182] S.-L. You, J. W. Kelly, *Organic Letters* **2004**, *6*, 1681.
[183] L.-h. Zhang, G. S. Kauffman, J. A. Pesti, J. Yin, *Journal of Organic Chemistry* **1997**, *62*, 6918.
[184] K. Nakayama, M. Endo, M. Fujitsuka, T. Majima, *Journal of Physical Chemistry B* **2006**, *110*, 21311.
[185] M. S. Macauley, G. E. Whitworth, A. W. Debowski, D. Chin, D. J. Vocadlo, *Journal of Biological Chemistry* **2005**, *280*, 25313.
[186] R. Grigg, M. A. B. Sarker, *Tetrahedron* **2006**, *62*, 10332.
[187] P. Loos, M. Riedrich, H.-D. Arndt, *Chemical Communications (Cambridge, United Kingdom)* **2009**, 1900.
[188] C. Baumgartner, C. Eberle, F. Diederich, S. Lauw, F. Rohdich, W. Eisenreich, A. Bacher, *Helvetica Chimica Acta* **2007**, *90*, 1043.
[189] N. A. Boland, M. Casey, S. J. Hynes, J. W. Matthews, M. P. Smyth, *Journal of Organic Chemistry* **2002**, *67*, 3919.
[190] F. Menges, A. Pfaltz, in *Solvias AG, Switz. .* **2005**, p. 68 pp.
[191] M. S. Manhas, W. H. Hoffman, B. Lal, A. K. Bose, *Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry (1972-1999)* **1975**, 461.
[192] P. M. T. Ferreira, H. L. S. Maia, L. S. Monteiro, J. Sacramento, *Journal of the Chemical Society, Perkin Transactions 1* **2001**, 3167.
[193] B. Nyasse, L. Grehn, U. Ragnarsson, H. L. S. Maia, L. S. Monteiro, I. Leito, I. Koppel, J. Koppel, *Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-Organic Chemistry* **1995**, 2025.
[194] B. Nyasse, L. Grehn, U. Ragnarsson, *Chemical Communications (Cambridge)* **1997**, 1017.
[195] Z. van, Anthoni W., F. Lopez, A. J. Minnaard, B. L. Feringa, *Journal of Organic Chemistry* **2007**, *72*, 2558.
[196] G. Ewin, J. O. Hill, *Australian Journal of Chemistry* **1979**, *32*, 441.
[197] M. J. Petersson, I. D. Jenkins, W. A. Loughlin, *Organic & Biomolecular Chemistry* **2009**, *7*, 739.
[198] K. Takeda, S. Yano, M. Sato, E. Yoshii, *Journal of Organic Chemistry* **1987**, *52*, 4135.
[199] J. B. Hendrickson, M. S. Hussoin, *Journal of Organic Chemistry* **1989**, *54*, 1144.
[200] J. B. Hendrickson, M. S. Hussoin, *Synlett* **1990**, 423.
[201] J. B. Hendrickson, in *Encyclopedia of Reagents for Organic Synthesis, Vol. 8* (Ed.: L. A. Paquette), Wiley, New York, NY, **1995**, pp. 5405.

[202] K. E. Elson, I. D. Jenkins, W. A. Loughlin, *Australian Journal of Chemistry* **2004**, *57*, 371.
[203] D. R. Williams, S. Patnaik, M. P. Clark, *Journal of Organic Chemistry* **2001**, *66*, 8463.
[204] S. Hanessian, T. J. Liak, B. Vanasse, *Synthesis* **1981**, 396.
[205] L. He, Q. Wang, G.-C. Zhou, L. Guo, X.-Q. Yu, *ARKIVOC (Gainesville, FL, United States)* **2008**, 103.
[206] P. C. Dedon, *Chemical Research in Toxicology* **2008**, *21*, 206.
[207] C. F. Garbers, J. A. Steenkamp, H. E. Visagie, *Tetrahedron Letters* **1975**, 3753.
[208] S. J. Coats, J. S. Link, D. J. Hlasta, *Organic Letters* **2003**, *5*, 721.
[209] F. Yokokawa, H. Sameshima, D. Katagiri, T. Aoyama, T. Shioiri, *Tetrahedron* **2002**, *58*, 9445.
[210] W. Liyanage, L. Weerasinghe, R. K. Strong, V. Del, Juan R., *Journal of Organic Chemistry* **2008**, *73*, 7420.
[211] P. Wipf, C. P. Miller, S. Venkatraman, P. C. Fritch, *Tetrahedron Letters* **1995**, *36*, 6395.
[212] S.-F. Lu, D.-M. Du, S.-W. Zhang, J. Xu, *Tetrahedron: Asymmetry* **2004**, *15*, 3433.
[213] J. Bergman, B. Pettersson, V. Hasimbegovic, P. H. Svensson, *Journal of Organic Chemistry* **2011**, *76*, 1546.
[214] D. R. Williams, P. D. Lowder, Y.-G. Gu, D. A. Brooks, *Tetrahedron Letters* **1997**, *38*, 331.
[215] O. Mitsunobu, M. Yamada, *Bulletin of the Chemical Society of Japan* **1967**, *40*, 2380.
[216] D. B. Dess, J. C. Martin, *Journal of the American Chemical Society* **1991**, *113*, 7277.
[217] S. Ballut, D. Naud-Martin, B. Loock, P. Maillard, *Journal of Organic Chemistry* **2011**, *76*, 2010.
[218] M. Zhao, J. Li, Z. Song, R. Desmond, D. M. Tschaen, E. J. J. Grabowski, P. J. Reider, *Tetrahedron Letters* **1998**, *39*, 5323.
[219] J. R. Casimir, L. Ettouati, J. Paris, *Letters in Peptide Science* **1998**, *5*, 13.
[220] Y. Jin, Y. Liu, Z. Wang, S. Kwong, Z. Xu, T. Ye, *Organic Letters* **2010**, *12*, 1100.
[221] J. B. Epp, T. S. Widlanski, *Journal of Organic Chemistry* **1999**, *64*, 293.
[222] R. Xu, Z.-H. Jiang, *Carbohydrate Research* **2008**, *343*, 7.
[223] S.-H. Lee, J. Yoon, K. Nakamura, Y.-S. Lee, *Organic Letters* **2000**, *2*, 1243.
[224] W. Cabri, *Tetrahedron Letters* **1996**, *37*, 4785.
[225] M. Primozic, M. Habulin, Z. Knez, *Journal of the American Oil Chemists' Society* **2005**, *82*, 543.
[226] N. Kaur, R. S. Dhillon, *Indian Journal of Chemistry, Section B: Organic Chemistry Including Medicinal Chemistry* **2001**, *40B*, 574.
[227] R. P. Singh, J. n. M. Shreeve, *Synthesis* **2002**, 2561.
[228] T. M. A. Elmaati, *Journal of Heterocyclic Chemistry* **2004**, *41*, 947.
[229] M. Dieguez, O. Pamies, *Chemistry--A European Journal* **2008**, *14*, 3653.
[230] G. Pattenden, N. J. Ashweek, C. A. G. Baker-Glenn, J. Kempson, G. M. Walker, J. G. K. Yee, *Organic & Biomolecular Chemistry* **2008**, *6*, 1478.
[231] M. Kirihara, T. Kambayashi, T. Momose, *Chemical Communications (Cambridge)* **1996**, 1103.
[232] U. Stelzer, F. Effenberger, *Tetrahedron: Asymmetry* **1993**, *4*, 161.

[233] F. Beaulieu, L.-P. Beauregard, G. Courchesne, M. Couturier, F. LaFlamme, A. L'Heureux, *Organic Letters* **2009**, *11*, 5050.
[234] A. L'Heureux, F. Beaulieu, C. Bennett, D. R. Bill, S. Clayton, F. La, Francois, M. Mirmehrabi, S. Tadayon, D. Tovell, M. Couturier, *Journal of Organic Chemistry* **2010**, *75*, 3401.
[235] R. F. Cunico, L. Bedell, *Journal of Organic Chemistry* **1980**, *45*, 4797.
[236] A. Winkel, R. Wilhelm, *Tetrahedron: Asymmetry* **2009**, *20*, 2344.
[237] X. Qi, X. Wang, L. Wang, Q. Wang, S. Cheng, J. Suo, J. Chang, *European Journal of Medicinal Chemistry* **2005**, *40*, 805.
[238] A. K. Boal, I. Guryanov, A. Moretto, M. Crisma, E. L. Lanni, C. Toniolo, R. H. Grubbs, D. J. O'Leary, *Journal of the American Chemical Society* **2007**, *129*, 6986.
[239] T. W. Hart, D. A. Metcalfe, F. Scheinmann, *Journal of the Chemical Society, Chemical Communications* **1979**, 156.
[240] L. A. Carpino, H. Imazumi, A. El-Faham, F. J. Ferrer, C. Zhang, Y. Lee, B. M. Foxman, P. Henklein, C. Hanay, C. Mügge, H. Wenschuh, J. Klose, M. Beyermann, M. Bienert, *Angew Chem* **2002**, *114*, 457.
[241] E. Frerot, J. Coste, A. Pantaloni, M. N. Dufour, P. Jouin, *Tetrahedron* **1991**, *47*, 259.
[242] W. Koenig, R. Geiger, *Chemische Berichte* **1970**, *103*, 788.
[243] K. Barlos, D. Papaioannou, D. Theodoropoulos, *International Journal of Peptide & Protein Research* **1984**, *23*, 300.
[244] M. Crisma, V. Moretto, F. Formaggio, C. Toniolo, *Zeitschrift fuer Kristallographie* **1999**, *214*, 766.
[245] M. Vlassi, G. Germain, K. Barlos, P. Mamos, L. S. Refaat, *Zeitschrift fuer Kristallographie* **1990**, *192*, 59.
[246] K. Yamada, S. Dong, G. Wu, in *J. Am. Chem. Soc, Vol. 122*, **2000**, pp. 11602.
[247] A. Steinschneider, D. Fiat, *Int J Pept Protein Res* **1984**, *23*, 591.
[248] E. Ponnusamy, U. Fotadar, A. Spisni, D. Fiat, *Synthesis* **1986**, *1*, 48.
[249] R. Goc, E. Ponnusamy, J. Tritt-Goc, D. Fiat, *Int J Pept Protein Res* **1988**, *31*, 130.
[250] A. Steinschneider, M. I. Burgar, A. Buku, D. Fiat, *Int. J. Peptide Protein Res.* **1981**, *18*, 324.
[251] K. Yamada, T. Yamazaki, M. Asanuma, H. Hirota, N. Yamamoto, Y. Kaiihara, *Chemistry Letters* **2007**, *36*, 192.
[252] K. Yamada, T. Shimizu, M. Tansho, T. Nemoto, M. Asanuma, M. Yoshida, T. Yamazaki, H. Hirota, *Magn Reson Chem* **2007**, *45*, 547.
[253] V. Theodorou, A. N. Troganis, I. P. Gerothanassis, *Tetrahedron Letters* **2004**, *45*, 2243.
[254] E. Ponnusamy, H. Eckert, D. Fiat, *Int J Pept Protein Res* **1988**, *32*, 21.
[255] J. Marecek, B. Song, S. Brewer, J. Belyea, R. B. Dyer, D. P. Raleigh, *Org Lett* **2007**, *9*, 4935.
[256] J. Bredenbeck, J. Helbing, R. Behrendt, C. Renner, L. Moroder, J. Wachtveitl, P. Hamm, *J Phys Chem* **2003**, *107*, 8654.
[257] J. A. Ihalainen, J. Bredenbeck, R. Pfister, J. Helbing, L. Chi, I. H. M. Stokkum, G. A. Woolley, P. Hamm, *Proc Natl Acad Sci USA* **2007**, *104*, 5383.
[258] P. Hamm, M. Lim, R. M. Hochstrasser, *Journal of Physical Chemistry B* **1998**, *102*, 6123.
[259] S. Woutersen, P. Hamm, *J. Phys.: Condens. Matter* **2002**, *14*, R1035.
[260] J. A. Ihalainen, B. Paoli, S. Muff, E. H. Backus, J. Bredenbeck, G. A. Woolley, A. Caflisch, P. Hamm, *Proc Natl Acad Sci U S A* **2008**, *105*, 9588.

[261] S. H. Shim, R. Gupta, Y. L. Ling, D. B. Strasfeld, D. P. Raleigh, M. T. Zanni, *Proc Natl Acad Sci U S A* **2009**, *106*, 6614.
[262] P. Mukherjee, A. T. Krumml, E. C. Fulmer, I. Kass, I. T. Arkin, M. T. Zanni, *J. Chem. Phys.* **2004**, *120*, 10215.
[263] P. Mukherjee, I. Kass, I. T. Arkin, M. T. Zanni, *Proc Natl Acad Sci U S A* **2006**, *103*, 3528.
[264] P. Mukherjee, I. Kass, I. T. Arkin, M. T. Zanni, *J Phys Chem B* **2006**, *110*, 24740.
[265] C. Fang, R. M. Hochstrasser, *J Phys Chem B* **2005**, *109*, 18652.
[266] C. Fang, A. Senes, L. Cristian, W. F. DeGrado, R. M. Hochstrasser, *Proc Natl Acad Sci U S A* **2006**, *103*, 16740.
[267] C. Fang, J. D. Bauman, K. Das, A. Remorino, E. Arnold, R. M. Hochstrasser, *Proc Natl Acad Sci U S A* **2008**, *105*, 1472.
[268] E. Ponnusamy, G. R. Jones, D. Fiat, *J. Labelled Compd. Rad.* **1987**, *24*, 773.
[269] H. Hollenstein, R. W. Schaer, N. Schwizgebel, G. Grassi, H. H. Guenthard, *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy* **1983**, *39A*, 193.
[270] R. C. Murphy, K. L. Clay, *Biomed Mass Spectrom* **1979**, *6*, 309.
[271] C. Gervais, R. Dupree, K. J. Pike, C. Bonhomme, M. Profeta, C. J. Pickard, F. Mauri, *J Phys Chem A* **2005**, *109*, 6960.
[272] G. Wu, S. Dong, *J Am Chem Soc* **2001**, *123*, 9119.
[273] M. S. Seyfried, B. S. Lauber, N. W. Luedtke, *Organic Letters* **2010**, *12*, 104.
[274] N. Wrobel, M. Schinkinger, V. M. Mirsky, *Anal Biochem* **2002**, *305*, 135.
[275] A. G. Cassano, V. E. Anderson, M. E. Harris, *Biochemistry* **2004**, *43*, 10547.
[276] J. M. Risley, R. L. Van Etten, *J. Am. Chem. Soc.* **1980**, *102*, 4609.

14. Acknowledgements

I would like to express my sincere thanks to:

Prof. Dr. Nathan W. Luedtke for giving me the chance to accomplish this thesis, and for his continued and generous support;

Prof. Dr. Nathaniel Finney for co-refereeing this work;
Prof. Dr. Stefan Bienz and *Prof Dr. John A. Robinson* for serving as committee members;

PD Dr. Laurent Bigler, *Urs Stalder*, *Jean-Christophe Prost*, and *Silvan Eichenberger* for measuring many mass spectra;
Nadja Bross and *Simon Jurt* for help concerning NMR experiments
PD Dr. Anthony Linden, *Peter Übelhart*, and *Sacha Blumentritt* for X-ray crystal structure determinations and data retrieval;

Prof. Dr. Heinz Heimgartner for his encouragement from the first attended lecture till the end of my thesis and for giving me the opportunity to learn from an outstanding personality;

Renate Brokamp, *Birgit Lauber*, *Sabrina Huber*, and *Fabian Roth* for carrying out some important reactions;

All my colleagues form the laboratory of the group of Prof. Dr. Nathan W. Luedtke: *Dr. Jawad Alzeer, Dr. Todor Anguelov, Dr. Alessandro Cecci, Dr. Anaëlle Dumas, Felicia Gray, Sarah Hentschel, Sabrina Huber, Laura Lander, Birgit S. Lauber, Guillaume Mata, Anne Neef, David Oesch, Raul Pereira, Joëlle Räber, Fabian Roth, Philippe Roth, Olivia Schmidt, Bala Yeshwanth Ram Vummidi, Niklas von Wolff,* for friendship, good working atmosphere and support during my (and their) stay;

All other friend whom I could meet during my eleven years at University of Zürich;

The *Swiss National Science Foundation* for financial support.

15. Curriculum Vitae

Name:	Seyfried
First Name:	Martin Samuel
Date and place of birth:	05.01.1980 in Zürich, Zürich
Place of origin	Zürich and Meilen

Education

1987 – 1993	Elementary school in Feldmeilen
1993 – 2000	Kantonsschule Hohe Promenade, Matura Typus A and B
2000 – 2005	Chemistry Studies at the University of Zürich, Diploma thesis "*Reaktionen von Benzylidenphenylrhodanin mit verschiedenen 1,3-Dipolen*" in the group of Prof. Dr. Heinz Heimgartner
2006	Diploma degree in Chemistry Minor subject: Biochemistry
Nov. 2006 – Okt. 2011	Dissertation at the Institute of Organic Chemistry of the University of Zürich under the supervision of Prof. Dr. Nathan W. Luedtke. Titel: "*Design and Synthesis of Planar New Telomestatin Analogs*".
Nov. 2006 – Okt. 2011	Assistant in practical courses and lectures in chemistry for chemistry and medicine students.

Publication list:

Sabrina M. Huber, Martin S. Seyfried, Anthony Linden and Nathan W. Luedtke
"Excitonic Luminescence of Hemiporphyrazines" *Inorg. Chem.* **2011**, in review.

Martin S. Seyfried, Birgit S. Lauber, and Nathan W. Luedtke
"Multiple-Turnover Isotopic Labeling of Fmoc- and Boc-Protected Amino Acids with Oxygen Isotopes"
Org. Lett. **2010**, *12*, 104-106.

M.S. Seyfried, A. Linden, G. Mlostoñ and H. Heimgartner
"Chemoselectivity of the Reactions of Diazomethanes with 5-Benzylidene-3-phenylrhodanine"
Helv. Chim. Acta **2009**, *92*, 1800-1816.

Alexander Dichtl, Martin Seyfried, Kai-Uwe Schoening
"A Novel Method for the Synthesis for *N*-Alkoxyamines Starting from Nitroxide Radicals and Ketones"
Synlett **2008**, *12*, 1877-1881.

M.S. Seyfried, A. Linden, G. Mlostoñ and H. Heimgartner
"Chemoselectivity of the [2+3]-Cycloaddition of Thiocarbonyl Ylides with 5-Benzylidene-3-phenylrhodanine" *Polish J. Chem.* **2006**, *80*, 1363-1376.